Pharmacodynamics of Levofloxacin

A New Paradigm for Early Clinical Trials

Sandra L. Preston, PharmD; George L. Drusano, MD; Adam L. Berman; Cynthia L. Fowler, MD; Andrew T. Chow, PhD; Bruce Dornseif, PhD; Veronica Reichl, RN; Jaya Natarajan, PhD; Michael Corrado, MD

Context.—One purpose of early clinical trials is to establish the appropriate dose of an antibiotic for phase 3 trials. Development of a relationship between the ratio of drug exposure to organism minimum inhibitory concentration (MIC) and therapeutic response early in the development process would allow an optimal choice of dose to maximize response.

Objective.—To prospectively quantitate the relationship between plasma levels of levofloxacin and successful clinical and/or microbiological outcomes and occurrence of adverse events in infected patients.

Design.—Multicenter open-label trial.

Setting.—Twenty-two enrolling university-affiliated medical centers.

Patients.—A total of 313 patients with clinical signs and symptoms of bacterial infections of the respiratory tract, skin, or urinary tract.

Main Outcome Measures.—Clinical response and microbiological eradication of pathogenic organisms.

Results.—Of 313 patients, 272 had plasma concentration-time data obtained. Of these, 134 patients had a pathogen recovered from the primary infection site and had an MIC of the pathogen to levofloxacin determined. These patients constituted the primary analysis group for clinical outcome. Groups of 116 and 272 patients, respectively, were analyzed for microbiological outcome and incidence of adverse events. In a logistic regression analysis, the clinical outcome was predicted by the ratio of peak plasma concentration to MIC (Peak/MIC) and site of infection (P<.001). Microbiological eradication was predicted by the Peak/MIC ratio (P<.001). Both clinical and microbiological outcomes were most likely to be favorable if the Peak/MIC ratio was at least 12.2.

Conclusions.—Levofloxacin generated clinical and microbiological response rates of 95% and 96%, respectively. These response rates included fluoroquinolone “problem pathogens,” such as Streptococcus pneumoniae and Staphylococcus aureus. Exposure to levofloxacin was significantly associated with successful clinical and microbiological outcomes. The principles used in these analyses can be applied to other classes of drugs to develop similar relationships between exposure and outcome. This pharmacokinetic modeling could be used to determine optimal treatment dose in clinical trials in a shorter time frame with fewer patients. This modeling also should be evaluated for its potential to improve outcomes (maximizing therapeutic response, preventing emergence of resistance, and minimizing adverse events) of patients treated with this drug.

JAMA. 1998;279:125-129

For editorial comment see p 159.

When investigating anti-infective agents, in addition to a measure of drug exposure, a measure of the potency of the drug for the pathogen infecting an individual patient is required. In developing the therapeutic response relationships, one may take both into account by forming ratios between measures of exposure (peak concentration, area under the plasma concentration vs time curve [AUC]) and measures of drug potency (minimum inhibitory concentration [MIC]) or by examining the time that drug concentrations remain above the MIC. Because there is a wide range of MICs in different organisms causing infection, the ratio will have a much broader range than the exposure variables (eg, peak, AUC) or MICs alone. Consequently, it may be easier to determine these exposure-effect relationships for anti-infective agents relative to other drug classes.

We conducted a multicenter, noncomparative trial to assess the safety and efficacy of levofloxacin for the treatment of a variety of community-acquired infections. Our objective was to prospectively link a measure of exposure to an outcome, such as clinical efficacy, microbiological efficacy, and/or development of an adverse event, with the hypothesis that the use of a combination of newer modeling methods would allow delineation of such relationships.

METHODS

A total of 313 adult patients (18 years or older), from 22 university-affiliated medical centers, with clinical signs and symptoms of bacterial infections of the respiratory tract, skin, or urinary tract that were of significant severity to re-
quire at least 3 days of intravenous antibacterial therapy were evaluated.

Inclusion criteria were presence of signs and symptoms of acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, community-acquired bacterial pneumonia, complicated or uncomplicated bacterial skin infection, acute pyelonephritis, or complicated urinary tract infection, and the ability to give informed consent. Exclusion criteria were (1) infection due to a levofloxacin-resistant organism; (2) requirement for additional systemic antibacterial therapy; (3) previous allergic or serious adverse reaction to a quinolone; (4) presence of a seizure disorder or unstable psychiatric disorder; (5) recent history of head trauma; (6) cystic fibrosis; (7) severe renal failure (creatinine clearance <0.33 mL/s) or oliguria (urine output <20 mL/h); (8) shock (supine systolic blood pressure <80 mm Hg) due to any cause or high likelihood of death during the course of the study; (9) hemoglobin level less than 80 g/L or platelet count less than 50 × 10^9/L; (10) human immunodeficiency virus infection and CD4 cell count 0.2 × 10^9/L or less, organ transplantation, or neutropenia (absolute neutrophil count <0.5 × 10^9/L); (11) FIO2 greater than 55 mm Hg; and (12) pregnant or nursing women.

### Drug Dosage and Administration

Patients with respiratory tract or skin infections received 500 mg of levofloxacin every 24 hours intravenously for at least 3 doses. Patients with complicated urinary tract infections or acute pyelonephritis received 250 mg intravenously every 24 hours for at least 3 doses. Patients with moderate renal impairment (creatinine clearance 0.33-0.83 mL/s as calculated by the method of Cockcroft and Gault) received 500 mg every 48 hours. No dosage changes were made for renally impaired patients receiving the 250-mg dose. Following the 3 intravenous doses, all patients were allowed to complete their course of therapy with oral levofloxacin, if medically appropriate.

The duration of therapy for community-acquired bacterial pneumonia, skin infections, and acute bacterial sinusitis was 10 to 14 days; acute exacerbation of chronic bronchitis, 5 to 7 days; and urinary tract infections, 7 to 10 days.

### Outcome Evaluation

Clinical response was determined by comparing the patient’s baseline signs and symptoms of infection with those after therapy. Patients’ response to therapy was classified as follows: (1) cure was defined as resolution of clinically significant signs and symptoms associated with admission (baseline) bacterial infection along with stability (no change) or improvement or resolution of x-ray findings; (2) improvement was defined as partial resolution of clinical signs and symptoms of admission (baseline) bacterial infection without further antibacterial treatment and stability (no change) or improvement or resolution of x-ray findings; (3) failure was defined as no response to therapy; and (4) indeterminate was defined as unable to evaluate because patient was unavailable for follow-up. Cure and improvement were both considered a successful response. Failure was considered an unsuccessful response. If a patient died of a cause other than infection, an impression of the status of the infection at the time of death was rendered.

All pathogens isolated from the appropriate specimens responsible for the admission diagnosis of bacterial infection were evaluated for microbiological response to treatment as follows: (1) Eradicated—eradication of admission pathogen in the posttherapy cultures. If a patient’s infection had improved to the point where no material was available for culture, the admission pathogen was presumed eradicated. (2) Persisted—continued presence of the admission pathogen in the posttherapy cultures. If the patient had a clinical failure and no posttherapy culture was taken while the patient was not receiving antibiotics, then the admission pathogen was presumed to persist. (3) Persisted with acquisition of resistance—continued presence of the admission pathogen in the posttherapy cultures with documented emergence of resistance. (4) Unknown—no test-of-cure culture available because patient was unavailable for follow-up. Eradicating and presumed eradicated were considered successful responses. Persisted, presumed persisted, and persisted with emergence of resistance were considered unsuccessful responses.

All patients were evaluated for treatment-emergent adverse events. A treatment-emergent adverse event was defined as an adverse event that was new in onset or aggravated in severity or frequency following administration of levofloxacin regardless of relationship to drug. However, investigators provided an assessment of the drug-relatedness of the adverse event.

### Pharmacodynamic Analysis

Pharmacodynamic parameters were analyzed using the NPEM2 program to obtain population pharmacokinetic parameters using a 1- and 2-compartment open model with first-order elimination from the central compartment.

Bayesian pharmacokinetic parameter estimates were then determined for each patient using the population parameters obtained from NPEM2 and using the “population of one” utility within this program. The individual Bayesian parameter estimates were then used in the simulation module of ADAPT II to allow calculation of an AUC and to simulate individual peak and trough concentrations for each patient. Other derived parameters included ratio of peak plasma concentration to MIC (Peak/MIC), AUC/MIC ratio, and time plasma concentrations of the drug that remained above the MIC (Time>MIC), as a fraction of the dosing interval.

Pharmacodynamic Analysis

Analysis of patient data included the categorical variables of sex, race, organism isolated (each species of organism was treated as a separate category; if more than 1 organism was present, the organism that was most resistant to levofloxacin by MIC was used in the analysis), site of infection, and occurrence of bacteremia, as well as the continuous variables of age, MIC of organism, and the derived pharmacokinetic parameters of peak, trough, AUC, MIC, AUC/MIC, and Time>MIC. These variables were analyzed with logistic regression using the LOGIT module of SYSTAT (Evanston, Ill) to evaluate their effect on clinical outcome. Patient outcomes classified as cured and improved were coded together (successful outcome), and those classified as failed were coded separately (unsuccessful outcome). Only patients with estimated pharmacokinetic parameters and an isolated organism were included in the analysis. This last requirement was a prospectively determined part of the analysis. Significance of the variable’s impact on the probability of a successful clinical outcome was measured by the log-likelihood ratio test. In this test, twice the log-likelihood difference of the expanded model from the constant-only model was determined and com-
pared against a $x^2$ distribution with $1 df$ or the appropriate number of degrees of freedom. An $\alpha$ of less than .05 was deemed significant. Predictor variables that were significant were tested in the same way for model expansion using the log-likelihood ratio test for the significance of model expansion, starting with the most significant predictor variable in the model and attempting to expand the model with other variables in their order of significance.

The patient data listed above was analyzed to examine the effect of predictive variables on microbiological outcome. Patient’s pathogens were classified as eradicated or persisted. Only patients with estimated pharmacokinetic parameters, an organism isolated, an MIC value, and data on organism eradication or persistence were included. Logistic regression was used as described previously.

Using logistic regression, 3 analyses were performed to compare adverse events of the central nervous system (CNS) (including psychiatric disturbances), gastrointestinal tract, and skin with sex, race, site of infection, age, peak and trough plasma concentrations, and AUC. These 3 systems were chosen because they had an adequate number of events to attempt logistic regression analysis. Only patients with estimated pharmacokinetic parameters were included (N=272). These analyses used only patients with treatment-emergent adverse events assessed by the investigator as definitely, probably, or possibly related to drug. All other patients were classified as having no adverse event for the particular system being analyzed.

Breakpoints of pharmacodynamic variables (eg, Peak/MIC ratio, AUC/MIC ratio) that divided patients into lower and higher probability groups for successful clinical and microbiological outcome were determined using Classification and Regression Tree (CART) analysis.

**RESULTS**

Of 313 patients, 272 were included in the pharmacokinetic analysis; 36 patients were excluded because of lack of plasma concentration data, 3 because of physiologically impossible plasma concentrations and known sample acquisition from the infusion line, and 2 because of known infusion time misspecifications. Of these 272 patients, 134 had clinical outcome determinations and an identified microorganism with a determined MIC. This group of 134 patients was used for the primary efficacy analysis in an attempt to link predictor variables to the probability of a successful clinical outcome. Of these patients, there were 7 clinical failures.

The 134 patients did not differ from the full population of 272 patients with regard to sex, race, age, or plasma concentrations of levofloxacin (peak and trough concentrations as well as the AUC).

For the microbiological outcome analysis, 116 patients had microbiological outcome determined and the data set indicated above for clinical outcome. This group of 116 patients formed the primary data set for the microbiological outcome analysis. Of these patients, 5 had persisting organisms.

For the adverse event analysis, all patients with pharmacokinetic parameters (N=272) were included regardless of whether a pathogen was identified. There were 8 skin adverse events (2.9%), 16 CNS adverse events (5.9%), and 31 gastrointestinal adverse events (11.4%). These treatment-emergent adverse events were all thought to be definitely, probably, or possibly related to drug by the investigators.

**Population Pharmacokinetic Modeling**

It was determined that a 2-compartment pharmacokinetic model best fit the data. The mean, median, and SD of the pharmacokinetic parameter values for the 2-compartment open model are presented in Table 1. Median values for each of the parameters agreed well with the means. These parameter values are similar to those estimated previously using data from a study of healthy volunteers. Mean peak concentration and AUC for a levofloxacin dose of 500 mg and dosing interval of 24 hours were $8.67\pm3.99$ µg/mL and $72.53\pm51.17$ µg·h/mL, respectively. In this analysis, a total of 1528 samples were analyzed (mean, 5.6 samples per patient). Patients switched to oral from intravenous therapy at an average of 3.5 days, with a median of 3 days; 67% (210/313) switched to oral therapy on day 4.

**Clinical Outcome Analysis**

Six variables were significant univariately in affecting the probability of a successful clinical outcome, including site of infection, which was analyzed as a categorical variable, as well as MIC, Peak/MIC ratio, AUC/MIC ratio, Time>MIC, and age, which were analyzed as continuous variables. Peak/MIC ratio, AUC/MIC ratio, and Time>MIC were virtually indistinguishable in their ability to alter the probability of a successful outcome (Table 2). This is understandable as, when examined, Peak/MIC and AUC/MIC ratios were highly correlated, with an $r$ value of 0.942 (Spearman rank correlation). Peak/MIC ratio and Time>MIC had a Spearman rank correlation of 0.665.

**Microbiological Outcome Analysis**

Univariately, 5 predictive variables significantly affected the probability of a successful microbiological outcome, as shown in Table 3. These predictors are the same as those selected for the clinical outcome analysis, along with AUC. When these were examined for model expansion, the final model selected by the log-likelihood ratio test included only Peak/MIC ratio plus AUC (Table 4). A competing final model included Peak/MIC ratio alone (Table 3). The simpler model was preferred because of its greater physiologic believability. The break point was 12.2 for Peak/MIC ratio. Microbiological eradication success rates
Pharmacodynamics of Levofloxacin—Preston et al

for patients achieving a Peak/MIC ratio of greater than 12.2 and 12.2 or less were 100% and 80.8%, respectively. Probability plots for successful microbiological outcome for Peak/MIC ratio and Peak/MIC ratio plus AUC are shown in Figure 2.

Adverse Events

No pharmacological (drug-related) predictive variables significantly affected the probability of occurrence of an adverse event when gastrointestinal, skin, and CNS systems were examined. However, the analysis of definite, possible, and possible toxic events demonstrated that the probability of a CNS adverse event was influenced by site of infection and the probability of a skin adverse event was influenced by race, specifically, patients of Hispanic origin, with 50% of the skin adverse events occurring in this group (Table 5).

**COMMENT**

The primary hypothesis of this study was that, by using newer mathematical modeling tools, it is possible to prospectively determine relationships between measures of drug exposure and measures of patient outcome in relatively small, multicenter clinical trials. We successfully linked a measure of levofloxacin exposure (Peak/MIC) to clinical outcome and microbiological outcome. We were unable to link direct measures of drug exposure (peak concentrations, trough concentrations, or AUC) to the occurrence of CNS, skin, or gastrointestinal adverse events, even though the number of adverse events in each system was greater than the number of clinical or microbiological failures, indicating that there was most likely a sufficient number of occurrences of the adverse events to detect an association, if one had existed.

In these analyses, it is clear that pharmacological variables, when seen relative to a measure of potency of drug for the pathogen in question (MIC), can have a powerful effect on clinical outcome and microbiological outcome. These influences are sometimes modulated by the primary infection site (P = .03 for clinical outcome). Clinical failures were more likely for skin and soft tissue infections relative to either the respiratory tract or the urinary tract. Of the 7 clinical failures, 4 were in skin and soft tissue, 3 were in pulmonary sites, and none were in the urinary tract, for observed failure rates of 16%, 3%, and 0%, respectively. When examined more closely, 3 of 4 failures in the skin sites were in patients with complicated skin and skin structure infections, mostly elderly or diabetic patients with ulcers. One could speculate that the breakdown of the vascular system in such a circumstance could decrease the penetration of drug to the primary infection site.

Of note, none of the species of organisms (n = 35) behaved differently in terms of influencing outcome. This finding was further supported when each species of organism for which there were 5 or more isolates were analyzed alone vs all other organisms. Again, no species was shown to behave significantly different with regard to the probability of a successful clinical outcome. Of the 134 patients, 21 (15.7%) had infections with Streptococcus pneumoniae and 15 (11.2%) were infected with Staphylococcus aureus. Overall, 58% of the isolates were accounted for by 5 different species. All 7 patients with clinical failures had a different causative microorganism.

A previous single-center, retrospective investigation by Forrest et al with the fluoroquinolone ciprofloxacin indicated that AUC/MIC ratio was most closely linked to outcome. In our analysis, AUC/MIC ratio was significant, but on a statisti-
In the microbiological outcome analysis, the same pharmacometric variables were linked to the probability of eradication. The AUC was used because of its statistical significance in this analysis; however, the estimate is negative, meaning that a higher AUC is associated with a lower probability of a successful microbiological outcome. We believe that the negative correlation with AUC may have been influenced by chance because the mean AUC in the group of 5 unsuccessful microbiological outcomes (organism persistence) was 106.54 µg·h/mL vs. 60.79 µg·h/mL in the successful outcome group. The mean AUC/MIC ratio in the unsuccessful outcome group was 65.38 vs. 712.6 in the successful outcome group. This implies the mean MIC was higher in those with microbiological failures. The patients in the unsuccessful microbiological outcome group had a geometric mean MIC of 2.64 vs. 0.25 in the successful outcome group, which would obviously lead to a decreased Peak/MIC ratio for the microbiological outcome group. Patients with successful outcomes tended to have lower overall AUCs, but higher Peak/MIC and AUC/MIC ratios. The explanation for the higher AUCs in the unsuccessful outcome group could be a function of patient status, as the mean age was 63 years vs. 41 years in the successful outcome group. Because the finding of a lower AUC being associated with an improved microbiological outcome is physiologically improbable and is likely related to patient status, we thought that the final model of Peak/MIC ratio alone was logically stronger.

The peak points for both clinical and microbiological outcome seen for Peak/MIC ratio (12:2:1) are consistent with the mathematical model of fluoroquinolone effect with maximal tissue penetration. The model of fluoroquinolone effect with maximal tissue penetration is consistent with levofloxacin in inflammatory fluid. Attainment of this ratio with levofloxacin increases the probability of successful clinical outcome and may potentially decrease the probability of emergence of resistance, as an organism must persist to emerge resistant. The observed association between the occurrence of CNS adverse events and site of infection (sinus) is most likely due to the nature of sinus infections (ie, headaches are frequently associated with sinusitis). The relationship between skin adverse events and Hispanic origin is unclear.

No relationships were demonstrated between measures of exposure and the probability of occurrence of treatment-emergent adverse events for any of the 3 systems examined (gastrointestinal, skin, CNS). Clearly, as there were reasonable number of adverse events observed, we must conclude that the occurrence of these adverse events is only weakly (if at all) linked to our measures of exposure to levofloxacin.

In summary, we have prospectively determined relationships between exposure to levofloxacin and the probabilities of successful clinical outcome and microbiological outcome. This prospective development of drug concentration effect relationships can serve as a template for such relationship development in other anti-infective agents and in other therapeutic drug classes. Such relationships will allow rational drug therapy, which may result in maximally efficacious and minimally toxic clinical outcomes for ill patient populations and may allow design of regimens to minimize the emergence of drug-resistant pathogens.

This work was supported in part by a grant from the R. J. Johnson Pharmaceutical Research Institute. Interested investigators may contact the corresponding author for more complete information on the mathematical methods used in this study.

### References