ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1991, p. 1413–1422
0066-4804/91/071413-10$02.00/0

Vol. 35, No. 7

Correlation between In Vitro and In Vivo Activity of Antimicrobial Agents against Gram-Negative Bacilli in a Murine Infection Model

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Received 2 November 1990/Accepted 3 May 1991

We studied the relationship between in vitro susceptibility tests (MICs, MBCs) and in vivo activity of tobramycin, pefloxacin, ceftazidime, and imipenem against 15 gram-negative bacilli from five different species in a murine thigh infection model. Complete dose-response curves were determined for each antimicrobial agent against each strain, and three parameters of in vivo activity were defined: maximal attainable antimicrobial effect (i.e., reduction in $\log_{10}$ CFU per thigh compared with untreated controls) at 24 h (E_{max}), total dose required to reach 50% of maximal effect (P_{50}), and total dose required to achieve a bacteriostatic effect (static dose). Pefloxacin demonstrated the greatest E_{max} ($P < 0.05$). Tobramycin was the most potent antimicrobial agent, as indicated by its having the lowest static dose/MIC ratio ($P < 0.002$). $\log_{10}$ P_{50}s and static doses correlated significantly with $\log_{10}$ MICs or MBCs for the 15 strains of each antibiotic ($P < 0.01$) except imipenem ($P > 0.50$). The greater potency of imipenem against the three *Pseudomonas aeruginosa* strains than against strains of the family *Enterobacteriaceae* ($P < 0.01$) explained this lack of correlation. A longer duration of postantibiotic effect for imipenem against *P. aeruginosa* ($P = 0.02$) contributed to its increased potency against these strains. We conclude that in vitro susceptibility tests correlated well with in vivo activity in this animal model and that variations in potency among the four antimicrobial agents could be explained by differences in pharmacokinetics or pharmacodynamic activity.

Many clinical and animal studies have examined the relationship between in vitro susceptibility testing and in vivo efficacy (1, 7, 10–14, 18, 19, 23, 28). However, the ability of MICs and MBCs to predict in vivo activity is still problematic, and lack of correlation between in vitro and in vivo data is a common finding (37). Whether this discrepancy is a true microbiologic phenomenon or is due to inappropriate methodology remains unclear (23).

Most animal studies have examined the relationship between MIC and in vivo efficacy by testing a single bacterial strain against a large number of antimicrobial agents with various MICs (10–13). Such studies provide confusing results, since corrections must be made among the antimicrobial agents for differences in protein binding, pharmacokinetics, and pharmacodynamic activity, such as rates of bactericidal activity and postantibiotic effects (PAEs). They do not account for differences between organisms with regard to pathogenicity and virulence. In clinical studies (1, 7, 14, 18, 24, 28), data have been analyzed only qualitatively (sensitive versus resistant, success versus failure, death versus survival). Since ultimate clinical outcome depends on many parameters besides in vitro susceptibility (4, 20, 23, 25), such qualitative results do not allow specific examination of the impact of MIC on in vivo activity of antibiotics. In this study, we used a thigh infection model in neutropenic mice to obtain accurate and reproducible quantification of in vivo antimicrobial activity (22, 34). A wide range of antibiotic doses was employed in order to describe a complete dose-response curve and to determine quantitative parameters of in vivo activity (i.e., maximal antibacterial effect [E_{max}], dose required to achieve 50% of E_{max} [P_{50}], and dose required to achieve a bacteriostatic effect [static dose]) for four antibiotics (tobramycin, pefloxacin, ceftazidime, and imipenem) against 15 strains of gram-negative species most commonly associated with severe infection in humans (4, 8, 25). We described the relationship between MICs or MBCs and parameters of in vivo activity for each antibiotic and compared in vivo efficacy among the four antimicrobial agents.

(Part of this research was presented at the 28th Interscience Conference of Antimicrobial Agents and Chemotherapy, Los Angeles, Calif., October 1988.)

MATERIALS AND METHODS

Bacteria, media, and antibiotics. The following 15 bacterial strains were used for these experiments: *Escherichia coli* ATCC 25922; *Klebsiella pneumoniae* ATCC 43816; *Pseudomonas aeruginosa* ATCC 27853; and the 12 clinical isolates *E. coli* UW B and CB 855; *K. pneumoniae* VA 1601 and UW A; *Serratia marcescens* VA 1, USC 1944, and USC 1946; *Enterobacter cloacae* VA 13, UW 2781, and UW 22798; and *P. aeruginosa* UW A and UW B. Microorganisms were grown, subcultured, and quantified in Mueller-Hinton broth (MHB) and agar (Difco Laboratories, Detroit, Mich.). Antibiotics used for MIC determinations and in vivo studies included ceftazidime (Smith-Kline/Beecham Laboratories, Philadelphia, Pa.), imipenem/cilastatin (Merck, Sharp and Dohme, West Point, Pa.), tobramycin (Eli Lilly and Company, Indianapolis, Ind.), and pefloxacin (Rhone-Poulenc, Monmouth Junction, N.J.).

In vitro studies. MICs and MBCs were determined by standard macrodilution techniques using geometric twofold serial dilutions in MHB (29). MHB was supplemented with Ca^{2+} (50 mg/liter) and Mg^{2+} (20 mg/liter) for tobramycin testing. Each tube contained 1 ml (500 μl of inoculum plus 500 μl of drug-containing MHB). The final inoculum was 1 ×

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10^5 to 5×10^5 CFU/ml. MIC endpoints were determined visually after incubation at 35°C for 18 to 24 h. MBC endpoints were determined by subculturing 100 μl from the first cloudy tube and from all clear tubes onto Mueller-Hinton agar. A 99.9% reduction in CFU per milliliter (<10 CFU per plate) was used for the MBC (29). Final results were expressed as geometric means of two to four determinations.

In vitro PAEs were determined for imipenem with the 15 study strains by using a log-phase inoculum of 10^7 CFU in order to reproduce the in vivo inoculum at the start of therapy. After a 2-h exposure at a concentration equal to four times the MIC for each isolate, cultures were diluted 1:1,000 in fresh MHB. Aliquots were sampled every 1 to 2 h for 8 h for organism quantification. Experiments were repeated two to three times on different days for each strain. The in vitro PAE was calculated according to the method of Craig and Gudmundsson (6).

Mouse preparation and infection. Six-week-old, specific-pathogen-free, female ICR/Swiss mice weighing 23 to 27 g (Harlan Sprague-Dawley, Madison, Wis.) were rendered neutropenic (<100 neutrophils per mm^3) by injecting cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, Ind.) intraperitoneally 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) before the experiment (34). Broth cultures of freshly plated bacteria were grown into log phase overnight to an optical density of 0.3 at 580 nm (Spectronic 88; Bausch and Lomb, Rochester, N.Y.). After a 1:10 dilution into fresh broth, 0.1 ml (approximately 10^6 CFU) was injected into each thigh of ether-anesthetized mice.

Antimicrobial treatment. Mice were treated for 24 h with cumulative total doses of tobramycin (0.5 to 512 mg/kg), pefloxacin (1.25 to 1,280 mg/kg), ceftazidime (0.58 to 2,400 mg/kg), or imipenem (0.58 to 1,200 mg/kg), administered subcutaneously in divided doses every 6 h. Two mice were used for each regimen. Five to seven serial fourfold increases in total dose were used for each drug-organism combination to achieve a range of drug concentrations in serum that extended from below to above the concentration found in humans and to describe the complete dose-response relationship from no effect to maximal effect. When a maximal effect was not apparent with doses administered every 6 h, drugs were also given every 2 h. Antibiotics were administered in 0.2-ml volumes beginning 2 h after thigh inoculation. Untreated control mice were sacrificed for organism quantification just after thigh inoculation ($n = 1$), just before drug treatment ($n = 2$), and after 24 h ($n = 2$).

After sacrifice, thighs were removed and homogenized (Polytron tissue homogenizer; Kinematica, Lucerne, Switzerland) in 10 ml of iced 0.85% saline. Duplicate aliquots were plated in four to five serial 10-fold dilutions for CFU determination. Efficacy was calculated by subtracting the $\log_{10}$ CFU per thigh of each treated mouse at the end of therapy from the mean $\log_{10}$ CFU per thigh of control mice just before therapy (0 h) and at the end of therapy (24 h).

Drug pharmacokinetics. Single-dose plasma pharmacokinetic studies were performed in thigh-infected mice with ranges of individual doses as follows: tobramycin, 2 to 128 mg/kg; pefloxacin, 4 to 320 mg/kg; ceftazidime, 6.25 to 600 mg/kg; and imipenem, 1.56 to 400 mg/kg. For each of the four to nine individual doses examined, one or two groups of three to four mice were sampled four to five times each at 10 to 30 min intervals over four to five half-lives. Blood obtained by retro-orbital puncture in heparinized capillary tubes (Fischer Scientific Co., Pittsburgh, Pa.) was centrifuged for 10 min, and plasma levels were determined by an agar-well microbiologic assay using standard media and methods (9). Standard samples were prepared in both buffer and pooled normal mouse serum. Indicator organisms and media were *E. coli* ATCC 25922 and antibiotic medium no. 1 (Difco) for ceftazidime and pefloxacin, *Staphylococcus aureus* ATCC 6538P and antibiotic medium no. 1 for imipenem, and *Bacillus subtilis* ATCC 6633 and antibiotic medium no. 5 for tobramycin. Pharmacokinetic constants (elimination rate constant, half-life, maximum concentration of drug in serum, volume of distribution, area under the curve [AUC], and peak level) were calculated by utilizing a one-compartment model with zero-order absorption and first-order elimination via nonlinear least squares techniques (MINSQ; MicroMath Inc., Salt Lake City, Utah). Pharmacokinetic constants were interpolated from values obtained in the actual studies for doses that had no kinetics determined.

Statistical analysis. A sigmoid dose-effect model (E_{max} model) was employed to characterize in vivo antimicrobial efficacy. The model is described by the equation:

$$E = (E_{max} \times D^n)/(P_{50}{}^n + D^n) \quad (1)$$

where E is the observed effect (reduction in $\log_{10}$ CFU per thigh compared with 24-h controls), D is cumulative 24-h dose, E_{max} is a measure of relative efficacy indicated by the maximum antimicrobial effect attributable to the drug, P_{50} is a measure of potency indicated by the 24-h dose producing 50% of E_{max}, and n is a function describing the slope (16, 32). All three parameters of the equation (E_{max}, P_{50}, and n) were calculated by using nonlinear least squares regression techniques (MINSQ). The predictive performance of the model has been evaluated and shown to be excellent (22). E_{max} was calculated from regimens of doses every 2 h when it could not be accurately estimated from 6-h dosing. When this was necessary, E_{max} was fixed as a constant value and P_{50} and n were calculated for regimens of doses every 6 h. R^2 (the square of the correlation coefficient) was used to estimate the fraction of the total variance in antimicrobial effect that could be attributed to its nonlinear regression with the 24-h total dose.

To allow more meaningful comparison of potency among antimicrobial agents, the dose of antibiotic required to achieve a bacteriostatic effect in the thigh at 24 h compared with at the start of therapy (static dose) was estimated from the following equation, derived from equation 1:

$$\log_{10} \text{static dose} = \frac{\log_{10}[E_s/(E_{max} - E_s)]}{n} + \log_{10} P_{50} \quad (2)$$

where E_s is the antimicrobial effect that equals the control growth of the study strain between 0 and 24 h.

The relationship between in vitro susceptibility data ($\log_{10}$ MIC, $\log_{10}$ MBC) and in vivo parameters of potency ($\log_{10}$ P_{50}, $\log_{10}$ static dose) was calculated by linear regression analysis (Minitab Statistical Package, University of Pennsylvania, Philadelphia, Pa.). R^2 was also used to estimate the fraction of the total variance in potency that could be attributed to its linear regression with in vitro susceptibility data.

The Kruskal-Wallis test was used to compare parameters of in vivo activity (E_{max}, P_{50}, static dose) and slopes (n) among the four antibiotics. The Mann-Whitney test was used to compare the efficacies of any two antibiotics and the efficacies of each against strains of the family *Enterobacteriaceae* with those against *P. aeruginosa*.

TABLE 1. Pharmacodynamic parameters for tobramycin against 15 gram-negative bacilli

Strain	MIC (μg/ml)	MBC (μg/ml)	E_{max}[a]	P_{50} (mg/kg ± SD)	Static dose (mg/kg ± SD)
S. marcescens					
VA 1	2	4	4.71 ± 0.05[b]	189 ± 21	224 ± 25
USC 1944	16	16	6.26 ± 0.23[b]	1,560 ± 621	819 ± 318
USC 1946	>256	>256	ND[c]	ND	ND
K. pneumoniae					
VA 1601	32	>32	ND	ND	ND
UW A	0.5	0.7	4.44 ± 0.31[b]	32.2 ± 10.6	8.8 ± 2.9
ATCC 43816	0.5	0.7	4.87 ± 0.02	15.1 ± 2.6	17.9 ± 3.1
E. cloacae					
VA 13	0.5	1	4.43 ± 0.15[b]	37.2 ± 14.5	14.1 ± 5.5
UW 2781	0.5	1.4	2.31 ± 0.27[b]	17.6 ± 8.5	13.2 ± 6.4
UW 22798	0.7	0.7	3.81 ± 0.09[b]	31.9 ± 7.2	28.5 ± 6.4
E. coli					
ATCC 25922	1	1	4.15 ± 0.15[b]	40.4 ± 2.9	32.4 ± 2.3
UW B	1	1	5.31 ± 0.46[b]	31.6 ± 6.9	40.4 ± 8.8
CB 855	4	8	4.13 ± 0.10	32.6 ± 4.0	109.9 ± 13.5
P. aeruginosa					
ATCC 27853	1	1	6.99 ± 0.07	65.0 ± 4.7	47.3 ± 3.4
UW A	128	>128	ND	ND	ND
UW B	2	2	6.77 ± 0.38[b]	293 ± 22	54.5 ± 4.0
Overall mean			4.85 ± 1.33	199 ± 499	117 ± 229

[a] Reduction from 24-h controls, $\log_{10}$ ± SD.
[b] E_{max} achieved with regimen of doses every 2 h.
[c] ND, not done.

RESULTS

MIC and MBC determinations. Geometric mean MICs and MBCs of tobramycin, pefloxacin, ceftazidime, and imipenem for the 15 study strains are presented in Tables 1 to 4. For the strains studied in vivo, MICs and MBCs varied 32- and 22-fold for tobramycin, 89- and 125-fold for pefloxacin, 45- and 128-fold for ceftazidime, and 16- and 22-fold for imipenem, respectively.

Pharmacokinetics. Pharmacokinetic profiles, shown in Table 5, were relatively dose independent for all drugs tested (with the exception of the highest doses of pefloxacin). Peak plasma levels were achieved within 15 min for all but maximal doses. Peak-to-dose ratios for each drug varied at most twofold. AUC-to-dose ratios increased only at the highest doses. Elimination from plasma was rapid (except for pefloxacin), with terminal half-lives ranging from 11.0 to 24.9 min. Imipenem had a shorter half-life than ceftazidime. Pefloxacin had a longer half-life than tobramycin, despite smaller AUC/dose and peak plasma level/dose ratios. This was due to the larger volume of distribution of pefloxacin (range, 1.5 to 4.5 liters/kg) compared with that of tobramycin (range, 0.35 to 0.39 liters/kg).

Organism growth in thighs. Organisms exhibited logarithmic growth prior to therapy in all control experiments. Thigh-infected control mice had 6.10 ± 0.18 (mean of all experiments ± standard deviation [SD]) $\log_{10}$ CFU per thigh just after organism inoculation, which increased to 6.90 ± 0.42 $\log_{10}$ CFU per thigh upon initiation of therapy. Organisms in control mice continued to grow an additional 2.29 ± 0.57 $\log_{10}$ CFU per thigh over 24 h. At 24 h, variation in $\log_{10}$ CFU per thigh between two control mice was <0.25 for 73%, <0.5 for 99%, and <1.0 for 100% in all experiments. Variation between two mice treated with any given regimen was similar, with 54% being <0.25, 83% <0.5, and 96% <1.0 $\log_{10}$ CFU per thigh.

Dose-response curves and maximal efficacy. Representative dose-effect relationships for tobramycin against *K. pneumoniae* ATCC 43816 and ceftazidime against *E. coli* ATCC 25922 are shown in Fig. 1. A total of 56 individual dose-effect curves were generated and analyzed. Four strains were too resistant (three to tobramycin, one to ceftazidime) to allow accurate determination of the complete dose-response curve. Correlation of values predicted from equations to observed data was very good, with R^2 values ranging from 0.785 to 0.990 for tobramycin, 0.810 to 0.994 for pefloxacin, 0.891 to 0.972 for ceftazidime, and 0.741 to 0.988 for imipenem.

An asymptotic relationship was observed between cumulative dose and efficacy as it approached E_{max} for each of the 56 dose-response curves, i.e., where severalfold further increases in antibiotic dose did not increase efficacy. The E_{max} could be accurately estimated 14 times by dosing every 6 h. For the remaining 42 antibiotic-strain combinations, a regimen of dosing every 2 h was necessary to accurately estimate the E_{max}. The slopes of the individual sigmoid dose-response curves did not differ significantly among the four antibiotics.

In vivo efficacy. As shown in Fig. 2, significant differences in E_{max} (reduction of mean $\log_{10}$ CFU per thigh ± SD) were noted among the four antibiotics. Overall, pefloxacin exhibited a greater E_{max} than each of the three other antibiotics ($P < 0.05$) and was the only antimicrobial agent able to sterilize infected thighs (2 of 15 strains sterilized for pefloxacin versus 0 of 41 strains for the other antimicrobial agents; $P > 0.10$). Statistically important differences in the cumulative 6-h doses required to achieve 50% of E_{max} (P_{50}) were also noted

TABLE 2. Pharmacodynamic parameters for pefloxacin against 15 gram-negative bacilli

Strain	MIC (μg/ml)	MBC (μg/ml)	E_{max}[a]	P_{50} (mg/kg ± SD)	Static dose (mg/kg ± SD)
S. marcescens					
VA 1	0.5	0.5	6.59 ± 0.05[b]	38.2 ± 6.7	24.7 ± 4.3
USC 1944	0.35	0.7	≥7.35[c]	83.8 ± 8.5	45.6 ± 4.6
USC 1946	8	11.3	6.85 ± 0.25[b]	556 ± 70	322 ± 41
K. pneumoniae					
VA 1601	0.25	0.5	≥8.10[c]	78.1 ± 12.2	30.2 ± 4.7
UW A	0.5	1	5.01 ± 0.23[b]	93.2 ± 25.6	28.1 ± 7.7
ATCC 43816	0.25	0.35	5.57 ± 0.16[b]	29.2 ± 1.7	27.4 ± 1.6
E. cloacae					
VA 13	0.25	0.25	4.23 ± 0.10	12.0 ± 0.9	14.2 ± 1.1
UW 2781	0.25	1	3.36 ± 0.20	18.7 ± 3.4	28.5 ± 5.2
UW 22798	0.25	0.35	6.27 ± 0.14[b]	25.7 ± 6.3	17.2 ± 4.2
E. coli					
ATCC 25922	0.09	0.125	6.68 ± 1.08	6.5 ± 1.9	4.1 ± 1.2
UW B	0.09	0.25	5.73 ± 0.16[b]	13.1 ± 4.7	36.8 ± 13.2
CB 855	0.25	1.4	5.15 ± 0.05[b]	15.5 ± 1.7	56.8 ± 6.2
P. aeruginosa					
ATCC 27853	2.8	2.8	7.60 ± 0.25	219 ± 19	181 ± 15
UW A	1.4	2.8	6.60 ± 0.23[b]	38.8 ± 5.2	50.6 ± 6.8
UW B	1.4	2.8	7.06 ± 0.70[b]	276 ± 16	219 ± 12
Overall mean			6.14 ± 1.30	72.4 ± 92	112 ± 98

[a] Reduction from 24-h controls, $\log_{10}$ ± SD.
[b] E_{max} achieved with regimen of doses every 2 h.
[c] Sterilization of the four thighs.

among the four antibiotics (mean P_{50} in micrograms per kilogram ± SD, 199 ± 449, 100 ± 149, 371 ± 571, and 275 ± 339 for tobramycin, pefloxacin, ceftazidime, and imipenem, respectively; $P < 0.00001$). The two β-lactam antibiotics were significantly less potent than tobramycin and pefloxacin when given at six-h dosing intervals ($P < 0.01$).

In order to compare the in vivo activity of the four antibiotics with a parameter not directly dependent on E_{max}, as is P_{50}, the cumulative dose of antibiotic given every 6 h which was required to achieve a bacteriostatic effect in the thighs at 24 h (static dose) was estimated for each antibiotic against the 15 strains (Tables 1 to 4). As demonstrated in Fig. 1, the static dose could be larger or smaller than the P_{50}. Once again, important differences were noted among the four drugs (mean cumulative static dose in micrograms per kilogram ± SD, 117 ± 229, 72.4 ± 92.3, 251 ± 278, and 230 ± 249 for tobramycin, pefloxacin, ceftazidime, and imipenem, respectively; $P < 0.00001$). When dosed every 6 h, β-lactam antibiotics required higher total doses than tobramycin or pefloxacin to achieve an in vivo bacteriostatic effect ($P = 0.01$).

Finally, to evaluate in vivo potency among antibiotics with a parameter that is corrected for differences in MIC, we calculated the static dose/MIC ratio for each antibiotic against each study strain. Important differences were noted among the four antibiotics (mean static dose/MIC ratio ± SD, 40.5 ± 24.4, 112 ± 97.6, 213 ± 252, and 456 ± 385 for tobramycin, pefloxacin, ceftazidime, and imipenem, respectively; $P < 0.03$). Non-β-lactam antibiotics were more potent than β-lactams ($P = 0.0004$). Tobramycin was more potent than the other three drugs ($P < 0.002$). Ceftazidime was more potent than imipenem when strains of *Enterobacteriaceae* were compared (mean static dose/MIC ratio ± SD, 227 ± 284 and 583 ± 369, respectively; $P = 0.0013$). For the three *P. aeruginosa* strains, imipenem had a lower static dose/MIC ratio than ceftazidime, but the difference was not statistically significant because of the small number of strains (mean static dose/MIC ratio ± SD, 48.2 ± 30.2 and 162 ± 64.6, respectively; $P = 0.08$).

In order to investigate the greater potency of tobramycin compared with that of pefloxacin, we calculated the AUC/MIC ratio of each antibiotic necessary to achieve the same in vivo activity. The $\log_{10}$ AUC/MIC necessary to achieve an in vivo bacteriostatic effect at 24 h was not significantly different for tobramycin and pefloxacin (mean $\log_{10}$ AUC/MIC ± SD, 2.11 ± 0.31 and 2.29 ± 0.32, respectively; $P > 0.20$).

Correlation between in vitro susceptibility data and parameters of in vivo efficacy. As shown in Fig. 3, $\log_{10} P_{50}$ correlated with $\log_{10}$ MIC for tobramycin ($R^2 = 0.689$, $P < 0.001$), pefloxacin ($R^2 = 0.745$, $P < 0.001$), and ceftazidime ($R^2 = 0.699$, $P < 0.001$). Significant correlations ($P < 0.01$) between $\log_{10} P_{50}$ and $\log_{10}$ MBC ($R^2 = 0.517$, 0.642, and 0.582 for tobramycin, pefloxacin, and ceftazidime, respectively) were also observed. For the 12 strains of the family *Enterobacteriaceae*, $\log_{10} P_{50}$ for imipenem correlated significantly with $\log_{10}$ MIC ($R^2 = 0.545$, $P < 0.01$) and $\log_{10}$ MBC ($R^2 = 0.599$, $P < 0.01$). However, when the three *P. aeruginosa* strains were included in the analysis, $\log_{10} P_{50}$ no longer correlated with either $\log_{10}$ MIC ($R^2 = 0.064$, $P > 0.30$) or $\log_{10}$ MBC ($R^2 = 0.140$, $P > 0.10$). $\log_{10}$ static doses for tobramycin, pefloxacin, and ceftazidime also exhibited significant correlation with $\log_{10}$ MICs ($R^2 = 0.891$, 0.694 and 0.738, respectively; $P < 0.001$) and $\log_{10}$ MBCs ($R^2 = 0.781$, 0.799, and 0.724, respectively; $P < 0.001$). Again, the $\log_{10}$ static dose for imipenem was significantly correlated with the $\log_{10}$ MIC and MBC for the strains of *Enterobac-*

TABLE 3. Pharmacodynamic parameters for ceftazidime against 15 gram-negative bacilli

Strain	MIC (μg/ml)	MBC (μg/ml)	E_{max}[a]	P_{50} (mg/kg ± SD)	Static dose (mg/kg ± SD)
S. marcescens					
VA 1	0.25	1.3	3.76 ± 0.06[b]	54.4 ± 9.3	265 ± 42
USC 1944	0.25	0.5	5.57 ± 0.67[b]	51.0 ± 10	31.4 ± 6.4
USC 1946	5.6	16	4.43 ± 0.05[b]	914 ± 119	1,020 ± 132
K. pneumoniae					
VA 1601	0.7	0.7	4.86 ± 0.20[b]	123 ± 29	153 ± 35
UW A	0.25	0.5	6.53 ± 0.63[b]	79.0 ± 23	14.9 ± 4.2
ATCC 43816	0.25	0.25	6.37 ± 0.55[b]	84.0 ± 13	52.3 ± 8.3
E. cloacae					
VA 13	64	>64	ND[c]	ND	ND
UW 2781	0.7	0.7	3.07 ± 0.13[b]	143 ± 39	61.6 ± 17
UW 22798	2	2	4.19 ± 0.19[b]	638 ± 98	492 ± 76
E. coli					
ATCC 25922	0.125	0.125	5.27 ± 0.02	13.3 ± 2.5	5.7 ± 1.1
UW B	0.35	1	5.48 ± 0.10	52.6 ± 8.8	59.0 ± 9.9
CB 855	4	16	4.52 ± 0.21	176 ± 22	383 ± 47
P. aeruginosa					
ATCC 27853	2	8	6.05 ± 0.32[b]	595 ± 125	177 ± 37
UW A	2	4	3.98 ± 0.23[b]	154 ± 33	414 ± 90
UW B	2	5.65	4.63 ± 0.33[b]	2,111 ± 735	383 ± 133
Overall mean			4.91 ± 1.02	371 ± 571	251 ± 278

[a] Reduction from 24-h control, $\log_{10}$ ± SD.
[b] E_{max} achieved with regimen of doses every 2 h.
[c] ND, not done.

TABLE 4. Pharmacodynamic parameters for imipenem against 15 gram-negative bacilli

Strain	MIC (μg/ml)	MBC (μg/ml)	E_{max}[a]	P_{50} (mg/kg ± SD)	Static dose (mg/kg ± SD)
S. marcescens					
VA 1	0.5	1	5.07 ± 0.03[b]	718 ± 113	760 ± 119
USC 1944	0.35	0.5	6.14 ± 0.12[b]	255 ± 45	155 ± 28
USC 1946	1	8	6.17 ± 0.38[b]	1,311 ± 83	866 ± 55
K. pneumoniae					
VA 1601	0.5	1	4.00 ± 0.21	82.8 ± 14.2	174 ± 30
UW A	0.5	1	4.79 ± 0.12[b]	477 ± 50	286 ± 30
ATCC 43816	0.5	0.5	5.05 ± 0.17[b]	149 ± 18	149 ± 18
E. cloacae					
VA 13	0.35	0.35	3.73 ± 0.29[b]	159 ± 32	101 ± 20
UW 2781	0.35	0.5	2.30 ± 0.11[b]	109 ± 45	83.5 ± 35
UW 22798	0.5	0.7	3.68 ± 0.11[b]	272 ± 74	273 ± 74
E. coli					
ATCC 25922	0.125	0.35	4.59 ± 0.23[b]	72.3 ± 6.2	45.6 ± 3.9
UW B	0.35	0.35	5.45 ± 0.06[b]	115 ± 21	138 ± 25
CB 855	0.25	0.35	4.57 ± 0.12	40.0 ± 7.8	203 ± 39
P. aeruginosa					
ATCC 27853	2	8	6.67 ± 0.48[b]	80.6 ± 7.9	32.8 ± 3.2
UW A	2	2.8	5.46 ± 0.33[b]	178 ± 23	153 ± 20
UW B	1	2	6.17 ± 0.07[b]	103 ± 23	52.0 ± 11
Overall mean			4.92 ± 1.17	275 ± 339	230 ± 249

[a] Reduction from 24-h control, $\log_{10}$ ± SD.
[b] E_{max} achieved with regimen of doses every 2 h.

TABLE 5. Pharmacokinetic parameters in serum following a single dose in neutropenic thigh-infected mice

Drug	Dose (mg/kg)	Half-life (min)	AUC/dose [(mg · h/liter)/(mg/kg)]	Peak/dose [(mg/liter)/(mg/kg)]
Tobramycin	2–128	15.6–20.3	0.69–1.23	1.34–1.99
Pefloxacin	4–320	50.0–83.6	0.37–0.78	0.15–0.38
Ceftazidime	6.26–600	20.8–24.9	0.52–0.92	0.81–1.38
Imipenem	1.56–400	11.0–13.3	0.42–0.46	0.96–1.02

teriaceae (R^2 = 0.616 and 0.581, respectively; $P < 0.01$), but not when the three *P. aeruginosa* strains were included in the analysis ($P > 0.50$). There was no statistically significant difference among the slopes of the regression lines of the four antibiotics ($P > 0.05$).

Figure 4 displays the relationship between $\log_{10}$ static dose and $\log_{10}$ MIC of all antibiotics against all 15 strains (R^2 = 0.362, $P < 0.0001$). Similar correlations ($P < 0.001$) between $\log_{10}$ static dose and $\log_{10}$ MBC (R^2 = 0.403), $\log_{10} P_{50}$ and $\log_{10}$ MIC (R^2 = 0.408), and $\log_{10} P_{50}$ and $\log_{10}$ MBC (R^2 = 0.384) were observed. Non-β-lactam antibiotics, when analyzed separately, exhibited a significant correlation between $\log_{10}$ static dose and $\log_{10}$ MIC (R^2 = 0.664, $P < 0.0001$). Although β-lactams also demonstrated a significant correlation whether or not the *P. aeruginosa* strains were included in the analysis, the correlation was less than was observed with non-β-lactams (R^2 = 0.336, $P < 0.01$). The slopes of the regression lines were not significantly different between non-β-lactams and β-lactams (slope ± SD, 0.77 ± 0.11 and 0.70 ± 0.19, respectively; $P > 0.05$). In contrast, the intercept was significantly higher for β-lactams than for non-β-lactams (intercept ± SD, 2.267 ± 0.089 and 1.737 ± 0.060, respectively; $P < 0.05$).

As shown in Fig. 3, imipenem appeared to be more potent against *P. aeruginosa* than against strains of the family *Enterobacteriaceae*. To investigate this discrepancy, we compared the static dose/MIC ratios against strains of *Enterobacteriaceae* and against *P. aeruginosa* for each antibiotic. No difference could be detected for tobramycin, pefloxacin, and ceftazidime ($P > 0.70$). In contrast, there was a significant difference for imipenem between the three strains of *P. aeruginosa* (mean ± SD, 48.2 ± 30.2) and the 12 strains of *Enterobacteriaceae* (558 ± 362; P = 0.01). We have previously shown in this model that the time that levels in serum exceeded the MIC was the most significant parameter determining efficacy for β-lactams against various gram-negative bacilli (22, 34). By using this parameter, imipenem required a shorter duration of time above MIC to achieve an in vivo bacteriostatic effect against the three *P. aeruginosa* strains (mean percentage of time ± SD, 22.7 ± 4.6) than against the 12 strains of *Enterobacteriaceae* (36.3 ± 3.4, $P < 0.01$). In order to explain this difference, we quantified the in vitro PAE of imipenem against each bacterial strain after a 2-h exposure at a concentration equal to four times the MIC. The duration in PAEs ranged from 0.0 to 1.0 h for strains of *Enterobacteriaceae* (0.0 to 0.1 h for *K. pneumoniae*, 0.0 to 0.3 h for *E. coli*, 0.4 to 0.5 h for *S. marcescens*, and 0.3 to 1.0 h for *E. cloacae*). In contrast, PAEs lasted from 0.8 to 2.5 h for *P. aeruginosa*. PAEs were significantly longer for *P. aeruginosa* (mean ± SD, 1.6 ± .85 h) than for strains of *Enterobacteriaceae* (0.3 ± 0.3 h, P = 0.02).

DISCUSSION

The nature of the relationship between in vitro susceptibility tests and in vivo activity of antimicrobial agents remains unclear. In humans, many uncontrolled parameters could explain possible discrepancies between in vitro susceptibility tests and clinical outcome (23). However, several studies have demonstrated a correlation between MICs and clinical outcome (1, 7, 14, 18, 24) when in vitro and in vivo data are analyzed as qualitative parameters.

Experimental animal models which allow standardization of infection and treatment regimens (2, 3) have been em-

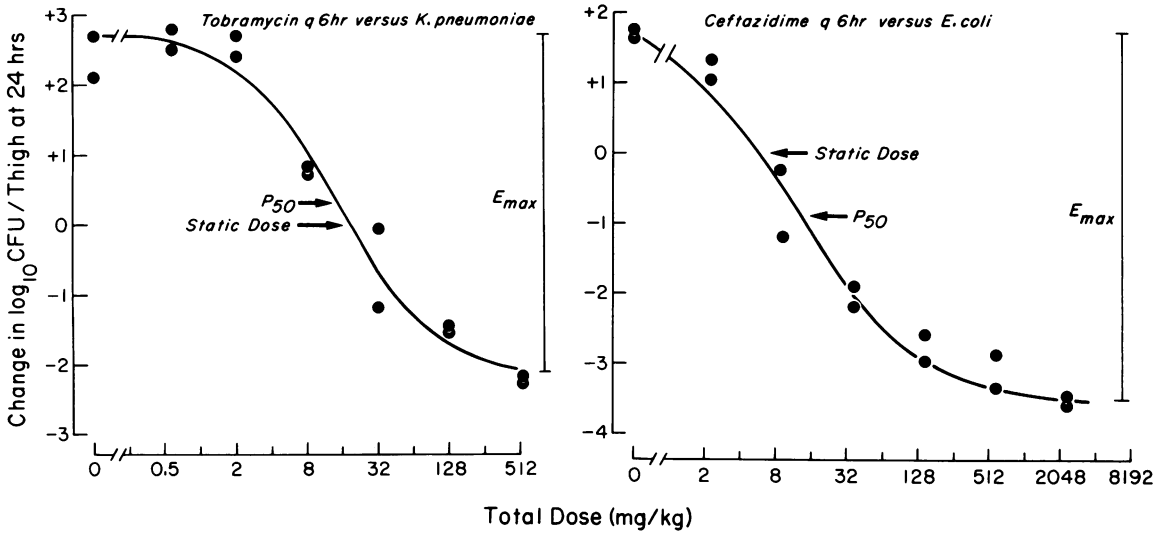


FIG. 1. Representative dose-effect relationships for tobramycin against *K. pneumoniae* ATCC 43816 (left panel) and ceftazidime against *E. coli* ATCC 25922 (right panel). Antibiotics were injected every 6 h into thigh-infected mice. Each point represents an individual mouse. Predicted dose-effect curves were generated from the three parameters calculated from equation 1 by nonlinear least squares regression techniques. R^2 values were 0.966 and 0.956 for tobramycin and ceftazidime, respectively. E_{max}, static dose, and P_{50} are indicated for each curve.

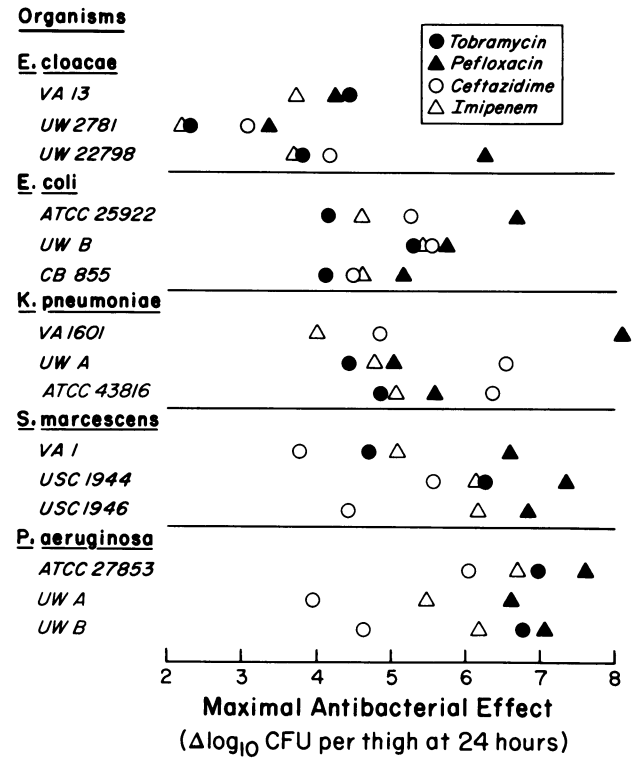


FIG. 2. E_{max} achieved by four antimicrobial agents against 15 gram-negative bacilli in a murine thigh infection model. Each symbol represents the reduction in $\log_{10}$ CFU per thigh at 24 h compared with that of untreated controls. Animals were injected every 2 h when the regimen of doses every 6 h did not allow accurate estimation of E_{max}. Pefloxacin exhibited a greater E_{max} than each of the three other antibiotics (reduction in mean $\log_{10}$ CFU per thigh ± SD, 6.14 ± 1.30 for pefloxacin versus 4.85 ± 1.33, 4.91 ± 1.01, and 4.92 ± 1.17 for tobramycin, ceftazidime, and imipenem, respectively).

ployed by many investigators to analyze the relationship between in vitro susceptibility tests and parameters of in vivo antimicrobial efficacy. However, results are conflicting since about one-third of such experiments found discrepancies between in vitro and in vivo data (37). One of the major limitations of such studies is the use of a single bacterial strain challenged with several antibiotics with different MICs and potentially different pharmacologic properties. Comparisons of antimicrobial agents must account for differences in pharmacodynamic and pharmacokinetic parameters or study only a single class of antimicrobial agents. Frimodt-Moller et al. (10), using a murine peritonitis model, demonstrated a significant correlation between MICs or MBCs and the 50% effective dose for 14 cephalosporins against one strain of *Streptococcus pneumoniae*. Tsuchiya et al. (31) compared the effect of seven cephalosporins against various gram-positive cocci and 13 gram-negative bacilli in a systemic murine infection. A significant correlation between MICs and 50% effective doses could be calculated from their data. In contrast, other investigators failed to show a correlation between MICs and in vivo activities of aminoglycosides even when they demonstrated such a correlation for β-lactams, probably because the range in MICs was too limited (12). No previous studies have examined the relationship between in vitro and in vivo activity of fluoroquinolones.

The methodology of our study differed markedly from those published previously by other investigators (10–13, 19, 31). For each antimicrobial agent studied, we used five to seven fourfold increases in total dose in order to describe a complete dose-response curve and to determine quantitative parameters of in vivo activity (E_{max}, static dose, and P_{50}). Dose-response curves were generated against 15 isolates from the five bacterial species most commonly associated with severe infections in humans (4, 8, 25). In this study, we have demonstrated a highly significant correlation between $\log_{10} P_{50}$ or static dose and $\log_{10}$ MIC or MBC for tobramycin, pefloxacin, and ceftazidime, and for imipenem against strains of *Enterobacteriaceae*. On the other hand, the activities of imipenem against the three *P. aeruginosa* strains were much greater than predicted by its MICs for these organisms compared with those for strains of the family *Enterobacteriaceae*. The significantly longer in vitro PAEs for imipenem against the three *P. aeruginosa* strains than against the 12 strains of *Enterobacteriaceae* might explain this discrepancy. We have previously demonstrated (15) in vivo PAEs for imipenem against two strains of *P. aeruginosa* (including strain ATCC 27853, used in the present study) ranging from 0.9 to 3 h, which is very close to our in vitro findings for the current *P. aeruginosa* strains (0.8 to 2.5 h). Quinolones and aminoglycosides also consistently produce prolonged PAEs with gram-negative bacilli (17, 26, 35). In contrast, ceftazidime and other β-lactams induce negligible or no PAEs against strains of *Enterobacteriaceae* and *P. aeruginosa* (35).

We achieved an in vivo E_{max} for all antibiotics. Pefloxacin exhibited a significantly greater E_{max} than tobramycin, ceftazidime, or imipenem and was the only antimicrobial agent able to sterilize infected mice. Greater in vivo activity of pefloxacin may be due to (i) relatively good in vitro activity against most gram-negative bacilli (27), (ii) an excellent pharmacokinetic profile, with a long elimination half-life and extensive extravascular distribution (36), and (iii) better penetration into phagocytic cells (30, 33).

As shown in Fig. 4, $\log_{10}$ MIC accounted for only 36% of the variance in $\log_{10}$ static dose when data with all four antibiotics were analyzed together. Valid comparison of in vivo activity among different antibiotics poses difficult problems. We have previously demonstrated that P_{50}s or the relative efficacy of a given total cumulative dose of aminoglycosides against *K. pneumoniae* was not affected by dosing intervals of between 1 and 6 h (22). In contrast, P_{50}s for ceftazidime and imipenem increased and the relative potency for any given total cumulative dose decreased proportionally with longer dosing intervals (22). Thus, it is not surprising that in this study, which was limited to regimens of dosings every 6 h, non-β-lactam antibiotics were more potent than β-lactams. By calculating the static dose/MIC ratio, we were able to compare in vivo potency and found that tobramycin was the most potent antimicrobial agent in this model, as indicated by its having the lowest static dose/MIC ratio.

We have previously demonstrated that $\log_{10}$ AUC in plasma is the most important parameter predicting efficacy of aminoglycosides (22, 33) and quinolones (21) against gram-negative bacilli in the thigh infection model. In this study, the $\log_{10}$ AUC/MIC ratio necessary to achieve a bacteriostatic effect was not significantly different for pefloxacin and tobramycin. However, pefloxacin, because of its larger volume of distribution, exhibited a smaller AUC/dose ratio than tobramycin. Thus, pefloxacin appeared to have lower potency (i.e., higher static dose/MIC ratio) than tobramycin, since a higher pefloxacin dose was required to

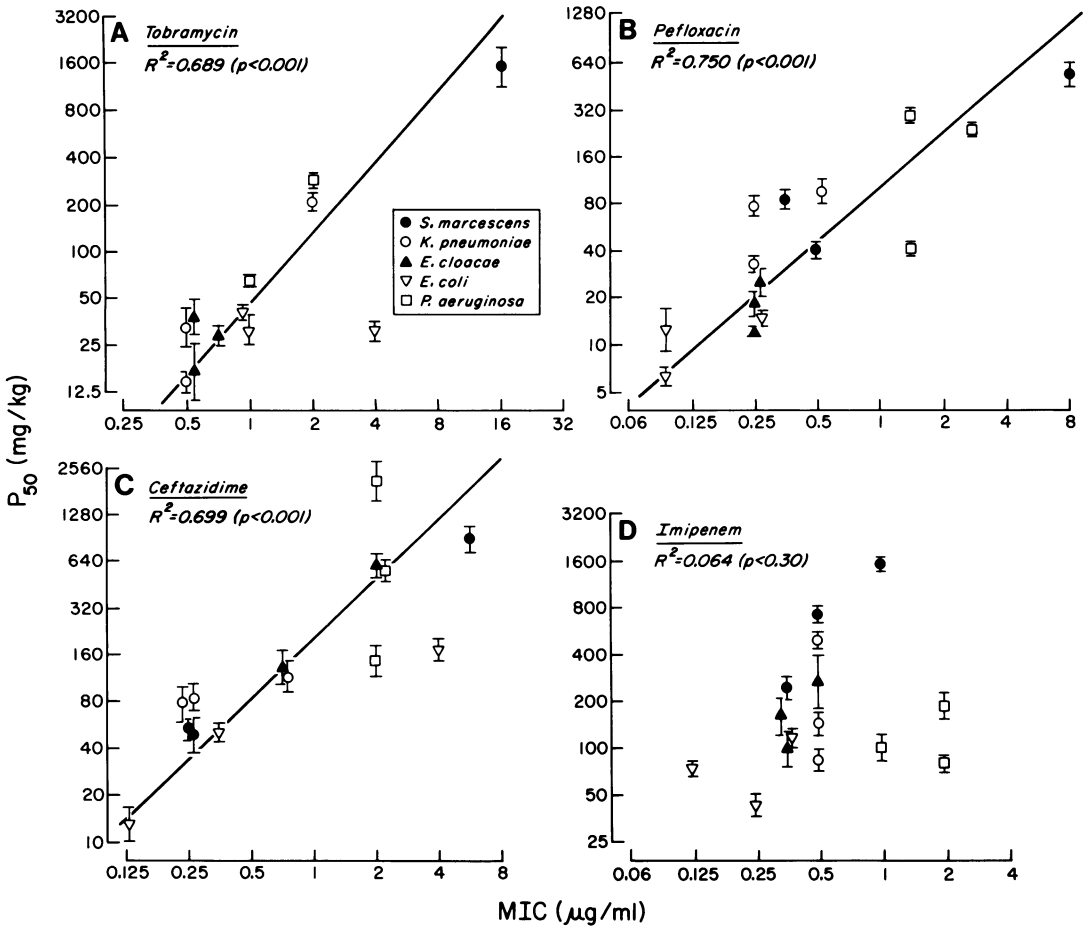
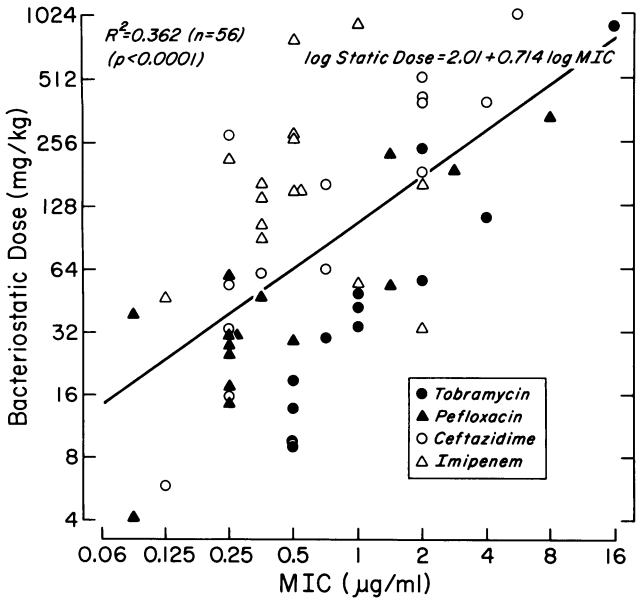


FIG. 3. Relationship between 24-h P_{50} (mg/kg, ± SD) and MIC (μg/ml) for tobramycin ($\log_{10} P_{50}$ = 1.689 + 1.065 $\log_{10}$ MIC) (A), pefloxacin ($\log_{10} P_{50}$ = 1.959 + 0.891 $\log_{10}$ MIC) (B), ceftazidime ($\log_{10} P_{50}$ = 2.291 + 0.936 $\log_{10}$ MIC) (C), and imipenem (no statistically significant correlation) (D).



achieve the same AUC/MIC ratio and in vivo activity observed with tobramycin.

For β-lactams, ceftazidime was more potent than imipenem against strains of *Enterobacteriaceae*. We have previously shown that the duration of time that antibiotic levels in serum exceed the MIC is the most important parameter predicting in vivo efficacy against gram-negative bacilli in this model (22, 34). The greater potency of ceftazidime could be due to its twofold longer half-life compared with that of imipenem, allowing longer periods of time during which ceftazidime levels in serum remain above the MIC. However, for *P. aeruginosa*, imipenem was more effective than ceftazidime. This may be due to the presence of a significant

FIG. 4. Correlation between 24-h static dose (mg/kg) and MIC (μg/ml) for the four antibiotics combined. Log_{10} static dose correlated with $\log_{10}$ MIC when β-lactams (open symbols; $\log_{10}$ static dose = 2.27 + 0.70 $\log_{10}$ MIC, R^2 = 0.336, $P < 0.01$) and non-β-lactams (closed symbols; $\log_{10}$ static dose = 1.74 + 0.77 $\log_{10}$ MIC, R^2 = 0.664, $P < 0.0001$) were analyzed separately. The slopes of the two regression lines were not different, but the intercept was significantly higher for β-lactams ($P < 0.05$), indicating that β-lactams required a consistently greater total dose than non-β-lactams to achieve a bacteriostatic effect for the same MIC.

PAE for imipenem compared with no PAE for ceftazidime against *P. aeruginosa* (5, 17, 35).

In conclusion, determination of dose-response curves generates parameters which allow an accurate evaluation of antimicrobial in vivo activity. Our study demonstrates that in vitro susceptibility tests exhibit a strong linear relationship with in vivo activity. Variations in potency among antimicrobial agents can largely be explained by differences in pharmacokinetics or pharmacodynamic activity.

ACKNOWLEDGMENTS

This research was funded by a Veterans Administration Merit Review Grant (W. A. Craig) and by grants from La Fondation pour La Recherche Medicale (B. Fantin) and Rhone-Poulenc, Inc. (W. A. Craig).

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