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Antibiotic Activity in Microbiological Media versus That in Human Urine: Comparison of Ampicillin, Ciprofloxacin, and Trimethoprim-Sulfamethoxazole

GLEN R. DROBOT,[1] JAMES A. KARLOWSKY,[1,2] DARYL J. HOBAN,[1,2] AND GEORGE G. ZHANEL[1,3,4]*

Department of Medical Microbiology, Faculty of Medicine,[1] and Faculty of Pharmacy,[3] University of Manitoba, and Departments of Medicine[4] and Clinical Microbiology,[2] Health Sciences Centre, Winnipeg, Manitoba, Canada R3A 1R9

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The activities of three antibiotics in both Mueller-Hinton broth (MHB) and pooled human urine were compared by using an in vitro pharmacodynamic model. Clinical and reference strains of *Escherichia coli* were exposed to antibiotics at concentrations achievable in human urine. The rate of bacterial killing (time to a reduction of 3 $\log_{10}$ CFU/ml) and the extent of bacterial killing at 24 h were examined. Between MHB and urine, there were no significant differences in the rate or extent of bacterial killing for both ampicillin and ciprofloxacin. For trimethoprim-sulfamethoxazole there was no significant difference in the extent of bacterial killing in urine compared with that in MHB ($P > 0.1$); however, there was a significant decrease in the rate of bacterial killing in urine compared with that in MHB ($P < 0.001$). We conclude that with ampicillin and ciprofloxacin, activity against *E. coli* in MHB is predictive of the effects in human urine. The activity of trimethoprim-sulfamethoxazole in MHB predicts the extent but not the rate of bacterial killing in human urine.

Antibiotic susceptibility testing, used to guide antibiotic therapy for patients with infectious diseases, is performed in standard microbiological media (e.g., Mueller-Hinton broth [MHB]). However, the correlation of antibiotic activity in microbiological media with that in human biological fluids such as urine is largely unknown. Like microbiological media, human urine is known to be an excellent medium for bacterial growth (1). It is important to determine the predictive value of antibiotic activity in vitro compared with that in in vivo situations. Currently, the antibiotics used to treat patients with urinary tract infections (UTIs) are chosen on the basis of culture and sensitivity tests performed in microbiological media and not human urine (7). We believe that it is important to compare antibiotic activity in microbiological media with that in human urine to establish the predictive value of antibiotic activity in vitro by using microbiological media.

The purpose of the study described here was to compare the activities of ampicillin, ciprofloxacin, and trimethoprim-sulfamethoxazole (TMP-SMX) against *Escherichia coli*, the most common pathogen in uncomplicated UTIs, in both microbiological media and human urine by using an in vitro pharmacodynamic model.

One reference strain and one clinical strain of *E. coli* were used: ATCC 25922 and an isolate (isolate 4549-1) from a patient with a UTI, respectively. *Bacillus subtilis* ATCC 6633 and *Klebsiella pneumoniae* ATCC 10031 were used for the antibiotic diffusion assay.

The antibiotics chosen for the study were ampicillin, ciprofloxacin, and TMP-SMX. These antibiotics are commonly used for the treatment of UTIs. They were prepared in stock solutions as described in National Committee for Clinical Laboratory Standards guidelines (7) and were injected as boluses into the in vitro model. The antibiotic concentrations used in the model were based on the achievable concentrations in urine after the administration of a standard dose (ampicillin, 2,000 μg/ml; ciprofloxacin, 350 μg/ml; TMP-SMX, 50 and 250 μg/ml) (6).

MHB supplemented with cations (Mg^{2+} and Ca^{2+}) or pooled human urine was used in the pharmacodynamic model. Urine was obtained from healthy laboratory technologists and students, pooled, stored at 4°C, centrifuged at 3,000 × *g* for 15 min to remove sloughed epithelial cells, prefiltered with a 1-μm-pore-size glass filter, and sterilized with a 0.45-μm-pore-size Millipore filter. Samples of the pooled urine were sent for biochemical analysis to confirm that the urine composition (e.g., osmolality) was within normal limits. Thymidine phosphorylase (0.1 U/ml) was added to the media for experiments performed with TMP-SMX. Colony counts were performed on blood agar plates. Neomycin assay agar and Mueller-Hinton agar were used for the antibiotic diffusion assay. The macrodilution method of determining MICs was performed in MHB or urine according to the guidelines of the National Committee for Clinical Laboratory Standards (7).

Antibiotic concentrations were determined by a microbiological agar diffusion assay by a method modified from that of Bennett et al. (2). This confirmed the predicted concentrations of the antibiotics on the basis of their half-lives ($t_{1/2}$s) in serum and their elimination rate constants. For ampicillin and trimethoprim, a suspension of *B. subtilis* spores (1 ml) was added to cooled Mueller-Hinton agar (125 ml). Trimethoprim was assayed separately and not in the presence of sulfamethoxazole. For ciprofloxacin, a suspension of *K. pneumoniae* (0.94 ml, 50% transmittance at 580 nm) was added to cooled neomycin assay agar (125 ml) (11). The limits of sensitivity for the bioassay were 0.05 μg/ml for ciprofloxacin and trimethoprim and 0.1 μg/ml for ampicillin.

The in vitro model stimulates a one-compartment open

* Corresponding author. Mailing address: MS6—Microbiology, Health Sciences Centre, 820 Sherbrook St., Winnipeg, Manitoba, Canada R3A 1R9. Phone: (204) 787-4902. Fax: (204) 787-4699.

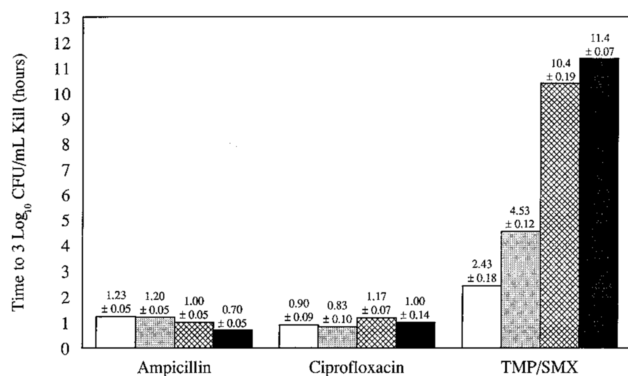

FIG. 1. Rate of bacterial killing in *E. coli*. Values above the bars represent the means ± SEMs of triplicate experiments. ▭, strain 25922 and MHB; ▒, strain 4549-1 and MHB; ▩, strain 25922 and urine; ■, strain 4549-1 and urine.

model with first-order excretion. This model has been used extensively to study antibiotic-bacteria pharmacodynamics and has been shown to correlate with the antibiotic effect in in vivo studies (3, 4). It consisted of a 650-ml central glass compartment connected via silicone and glass tubing to two reservoirs: a flask containing drug-free medium and a waste container. The flow rate (F), which was determined by the formula $F = V \times 0.693/t_{1/2}$ (where V is the volume of the glass chamber, and $t_{1/2}$ is the half-life of the antibiotic in serum), was maintained with a peristaltic pump. The temperature inside the chamber was maintained at 37°C with a magnetic stir bar and a hot plate. All components were sterilized by autoclaving at 121°C for 15 min. To ensure sterility, a 1-ml sample from the model was plated onto blood agar. After equilibration to 37°C, an inoculum of exponential-phase *E. coli* was injected into the central chamber to give a final inoculum of approximately 10^7 CFU/ml. After an equilibration phase (≈45 min), the pump was started and antibiotic was injected. Experiments were performed without the addition of antibiotic for each calculated flow rate to obtain growth controls. At appropriate time intervals, samples were extracted with a syringe, diluted, and plated in 100-μl amounts. Plate counts of viable bacteria were obtained after incubation at 37°C for 18 to 24 h. All experiments were performed in triplicate.

To avoid the effect of antibiotic carryover, extracellular antibiotic was either inactivated (ampicillin) or removed by washing (ciprofloxacin). For TMP-SMX no removal measures were required, because the TMP-SMX demonstrated no carryover effect when it was compared with the control. For ampicillin, 1 U of penicillinase (*E. coli* 205 TEM R^+ 566; Sigma Chemical Company) was added to the sample and was allowed to stand for 2 min. For ciprofloxacin, the samples were microcentrifuged and were washed twice with normal saline to remove the antibiotic. No significant reduction in bacterial counts occurred as a result of washing.

For statistical analysis the three antibiotics plus a growth control, two strains, and two media produced a 4-by-2-by-2 factorial experimental design. The experimental group means for each time point are shown in the kill curves (see Fig. 1 and 2). The rate of bacterial killing (defined as a reduction of ≥3 $\log_{10}$ CFU/ml) and the extent of bacterial killing ($\log_{10}$ CFU per milliliter assessed at 24 h) were determined. The means and standard errors of the means (SEMs) were calculated. Since, by definition, the increase for the growth control was only in the number of CFU per milliliter these data were excluded from the analysis of the rate of bactericidal effect to produce a 3-by-2-by-2 design. One-way and factorial analyses of variance were conducted on these data by using the Number Cruncher Statistical System software package (NCSS, version 5.03). Post hoc comparisons were analyzed by Scheffé's method.

Determination of the MICs of ampicillin (8 to 16 μg/ml) and TMP-SMX (0.125 μg/ml for trimethoprim) were not influenced by urine. For ciprofloxacin, MICs increased four- to eightfold in urine (MIC, 0.125 μg/ml) compared with that in MHB (MIC, 0.016 μg/ml). Susceptibility data were not different between the two strains of *E. coli.*

The microbiological agar diffusion assay confirmed that appropriate antibiotic concentrations were achieved inside the in vitro model and thus simulated standard oral doses of antibiotics (ampicillin, 2,000 μg/ml; ciprofloxacin, 350 μg/ml; TMP-SMX, 50 and 250 μg/ml, respectively). There was less than 10% variability between trials for each experimental group (data not shown).

The rates of bacterial killing are plotted in Fig. 1. All three antibiotics proved to be bactericidal. Figure 2 described the extent of bacterial killing at 24 h. For ampicillin there was no significant difference in either the rate or the extent of bacterial killing between the reference and clinical strains of *E. coli* or between MHB and human urine ($P > 0.10$ for all comparisons). The rate or extent of bacterial killing by ampicillin was not significantly different from that of the growth control at 24 h in either media (Fig. 2). The rate and extent of bacterial killing by ciprofloxacin was not significantly different between the reference and clinical strains of *E. coli* or between MHB and human urine ($P > 0.10$ for all comparisons). Ciprofloxacin eliminated all bacterial cells in both media by 6 h, and no regrowth was noted even at 24 or 48 h (Fig. 2). This rapid and extensive bacterial killing with ciprofloxacin occurred even in urine, despite the four- to eightfold increased MICs in urine. For TMP-SMX, the rate of killing of clinical bacterial strain 4549-1 was slower than that of ATCC 25922 in both MHB and urine ($P < 0.001$) (Fig. 1 and 3A). Urine significantly increased the time to the bactericidal effect of TMP-SMX ($P < 0.001$). Exposure to TMP-SMX caused significantly lower viable counts at 24 h compared with the counts in the growth controls; however, no differences between media or strains were observed ($P > 0.10$) (Fig. 2). Figures 3A and B illustrate how the rate of bacterial killing was reduced in urine compared with that in MHB; however, the extent of killing was unaffected.

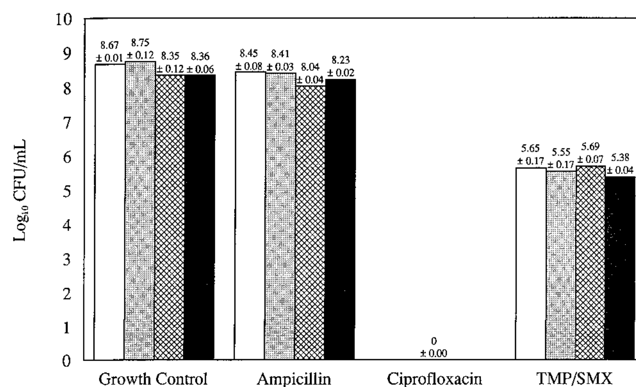

FIG. 2. Extent of bacterial killing at 24 h in *E. coli*. Values above the bars represent means ± SEMs of triplicate experiments. ▭, strain 25922 and MHB; ▒, strain 4549-1 and MHB; ▩, strain 25922 and urine; ■, strain 4549-1, and urine.

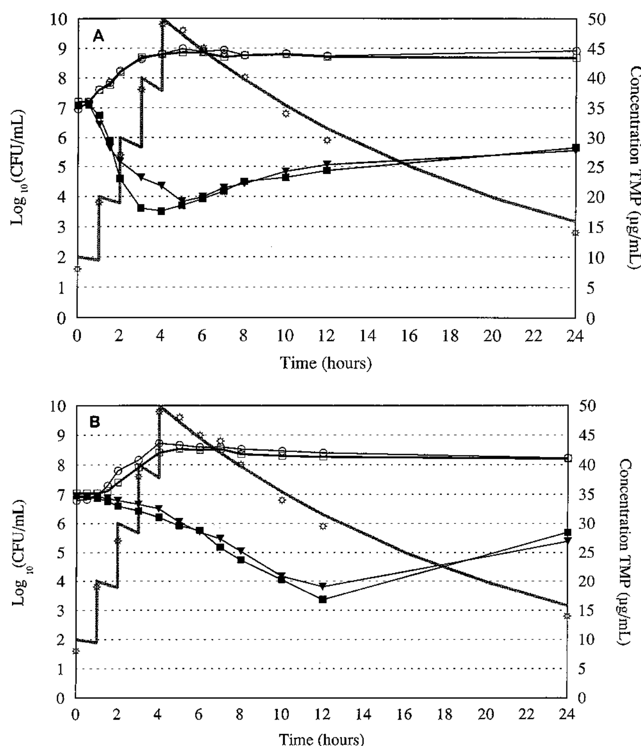

FIG. 3. (A) Time-kill curves for *E. coli* with TMP-SMX in MHB (A) and urine (B). All points are means of triplicate experiments. □, strain 25922; ○, strain 4549-1; ■, strain 25922 with TMP-SMX; ▼, strain 4549-1 with TMP-SMX; —, expected antibiotic concentration; ☼, observed antibiotic concentration.

Treatment of infection is guided by the results of culture and sensitivity tests performed in microbiological media such as MHB. The correlation between the rate and extent of killing in microbiological media and those in biological fluids such as urine is largely unknown. Using the in vitro pharmacodynamic model, we have shown that there is no difference in the rate and extent of killing with ampicillin in MHB or urine (maximum concentration of drug/MIC, 125 to 250). Despite a four- to eightfold increase in the MICs of ciprofloxacin in urine compared with that in MHB, ciprofloxacin also showed no difference in the rate or extent of bacterial killing between MHB and urine. This was likely due to the high concentration of ciprofloxacin in urine that we used. This high concentration produces very large maximum concentration of drug/MIC ratios (>2,800) (12). However, we found a significant difference in the rate of killing between MHB and urine for TMP (maximum concentration of drug/MIC, 400 for TMP. Thus, we conclude that the predictive value of the activity of TMP-SMX in MHB for that in urine is poor with regard to the rate of killing.

The macrodilution method for MIC determinations relies on visible growth at a fixed time (16 to 20 h) after inoculation; thus, it is a measure of the extent of growth. Since the MICs of TMP-SMX for the two strains of *E. coli* were not significantly different between MHB and urine, it is reasonable that no change in the extent of killing would be observed. Indeed, the extent of killing by TMP-SMX at 24 h did not change in urine compared with that in MHB. Nevertheless, some factor must be influencing the rate of killing by TMP-SMX in urine. No published data are available to explain this observation. There is a difference in pH between these media: MHB has a pH of 7.4, while pooled urine has a pH of 6.5. Upon the acidification of MHB (with HCl) to pH 6.5 and comparing it with urine at pH 6.5, the rate and extent of killing by TMP-SMX were very similar in MHB and urine. Since uptake of antibiotic into *E. coli* is dependent on the amount of uncharged antibiotic, it is possible that the relative acidity of urine (and acidified MHB [pH 6.5]) caused the amino groups of TMP and SMX to become positively charged. This increased amount of charged antibiotic likely leads to reduced uptake into bacterial cells. Reduced uptake of TMP-SMX would result in less antibiotic at the target site and reduced bacterial killing.

Uncomplicated UTI may be treated with a single dose of antibiotic (8, 10, 13). Philbrick and Bracikowski (10) calculated the cure rates for single-dose therapy for amoxicillin-ampicillin (69%) and TMP-SMX (87%). Pefloxacin, a fluoroquinolone like ciprofloxacin, has been studied as a single-dose agent and demonstrated a cure rate of 95% (9). The data obtained in the in vitro pharmacodynamic model may explain the in vivo situation with respect to these antibiotics. Specifically, the extent of growth at 24 h may predict clinical cure rates. In our model, because of its short $t_{1/2}$ (≈1 h), the level of ampicillin fell to sub-MICs in 10 h and *E. coli* subsequently regrew to the same extent as growth controls. In humans, ampicillin rapidly accumulates in the urine. With voiding, ampicillin is rapidly excreted from the body. The low maximum concentration of drug/MIC ratio (125 to 250) along with the lack of a sustained ampicillin concentration in the urine may explain the high microbiologic and/or clinical failure rates with single-dose ampicillin-amoxicillin therapy (10). Ciprofloxacin and TMP-SMX, with their high maximum concentration of drug/MIC ratios of >2,800 and 400 for ciprofloxacin and trimethoprim, respectively, along with their long $t_{1/2}$s (≈4 and 12 h, respectively) are both maintained at concentrations above the MIC for more than 24 h in the pharmacodynamic model, resulting in complete bacterial eradication with ciprofloxacin and a significantly greater extent of bacterial killing at 24 h than that of the growth control for both antibiotics. This may explain the higher cure rates of single-dose therapy with ciprofloxacin and TMP-SMX compared with that of ampicillin (8–10). We should state that although our data may help to explain cure rates after single-dose therapy, they cannot be used to extrapolate cure rates to the preferred 3-day therapy.

Perhaps in the treatment of UTIs the rate of bacterial killing is not a critical pharmacodynamic parameter affecting clinical cure and, thus, it is clinically insignificant that TMP-SMX demonstrates a reduced rate of bacterial killing in the urine compared with that in MHB. This situation is unlike that in the treatment of meningitis, in which time to bacterial eradication from the cerebrospinal fluid is an important pharmacodynamic parameter affecting patient outcome (5).

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