ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1991, p. 2658–2661
0066-4804/91/122658-04$02.00/0

Vol. 35, No. 12

Quantitation of Postantibiotic Effect by Measuring CO_2 Generation of Bacteria with the BACTEC Blood Culture System

MAGNUS GOTTFREDSSON, HELGA ERLENDSDOTTIR, AND SIGURDUR GUDMUNDSSON*

Department of Internal Medicine and Clinical Microbiology Laboratory, Borgarspitalinn, 108 Reykjavik, Iceland

Received 30 January 1991/Accepted 3 October 1991

The duration of the postantibiotic effect (PAE) determined by bacterial CO_2 production measured by using the BACTEC NR 730 blood culture system was compared with PAEs determined by standard viability counting. PAEs for *Staphylococcus aureus* after exposure to dicloxacillin, vancomycin, rifampin, gentamicin, and ciprofloxacin and for *Escherichia coli* after exposure to ampicillin, gentamicin, and ciprofloxacin were quantitated by the two methods, and an excellent correlation ($r = 0.93$) was demonstrated. The difference in the PAE durations determined by the two methods was 0.1 ± 0.4 (mean ± standard deviation) h. Thus, the BACTEC CO_2 generation method provides a simple, alternate way of determining the PAE in vitro.

The clinical significance of the postantibiotic effect (PAE) pertains primarily to the impact it may have on the design of antimicrobial dosing regimens in clinical practice (3), where antimicrobial agents inducing long PAEs against the offending organism may be administered with longer dosing intervals than before without loss of efficacy and even with a lower frequency of adverse reactions (15, 16).

The most widely used method for determining the PAE in vitro is by exposing the organism to an antimicrobial agent for a limited time and, after removal of drug, monitoring regrowth by serially measuring viable counts (3). Other methods have been used to quantitate the PAE, such as optical density (1, 2, 12, 13) and measurements of intracellular ATP (6–8, 11, 17), electrical impedance (9), and conductance (5) in bacterial cultures. The correlation between these methods and the rather time-consuming standard method has been variable, and some of the alternative methods require equipment that is usually not part of the standard armamentarium found in general clinical microbiology laboratories. Therefore, a simple alternative is needed. If the PAE is to be applied to specific clinical situations, determination of the PAE needs to be simple, require as little technician time as possible, and utilize technology already present in most laboratories.

In this regard, we examined CO_2 generation by bacteria as measured by the BACTEC NR 730 blood culture system (Becton Dickinson and Co., Sparks, Md.) for determination of the PAE and compared it directly with viable counting.

The BACTEC blood culture system detects bacterial CO_2 by infrared spectroscopy of gas aspirated from the headspace of a blood culture vial. CO_2 absorption measurement by spectrophotometer is expressed in terms of a growth value (GV), which is a unitless measurement derived from a comparison made by the system of the amount of CO_2 present in the vial headspace with the amount of CO_2 in aerobic (2.5% CO_2) or anaerobic (5.0% CO_2) reference culture gas. The manufacturer recommends that a GV of 30 be used as a cutoff for subculturing a blood culture vial. The bacterial viable count corresponding to this number is variable, ranging from 10^6 to 10^8 CFU/ml, and depends on the bacterial species and the pH, oxygenation, and temperature of the medium, etc. (4).

The test organisms employed in this study were two strains of *Staphylococcus aureus* (ATCC 25923 and clinical strain B5663) and two strains of *Escherichia coli* (ATCC 25922 and clinical strain B5756). The clinical strains were obtained from the Clinical Microbiology Laboratory, Borgarspitalinn, Reykjavik, Iceland. The organisms were stored at −20°C until testing. Prior to each experiment, the organisms were grown overnight and adjusted by a 0.5 McFarland standard to an inoculum of ~10^7 CFU/ml in the logarithmic phase.

The antimicrobial agents employed were obtained from the following manufacturers: dicloxacillin, Bristol Sermoneta, Latuia, Italy; ampicillin, Astra Läkemedel, Södertälje, Sweden; vancomycin, Eli Lilly & Co. Ltd., Indianapolis, Ind.; gentamicin, Roussel Laboratories Ltd., Uxbridge, United Kingdom; rifampin, CIBA-GEIGY AG, Basel, Switzerland; and ciprofloxacin, Bayer AG, Leverkusen, Germany. The antibiotics were diluted into solutions as recommended by the manufacturer to the desired concentration for each experiment. MICs were determined by standard methods in microtiter plates by using inocula of 5×10^5 to 1×10^6 CFU/ml (10).

The *S. aureus* strains were exposed, for 1 h in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) at 35.5°C, to dicloxacillin (in concentrations of four and eight times the MIC), vancomycin (two and four times the MIC), rifampin (two and four times the MIC), gentamicin (the MIC and twice the MIC), and ciprofloxacin (the MIC and twice the MIC). The *E. coli* strains were similarly exposed to ampicillin (four times the MIC), gentamicin (two and four times the MIC), and ciprofloxacin (two and four times the MIC).

Drug removal after exposure was accomplished by a 10^{-2} dilution of exposed and unexposed control cultures into fresh prewarmed BACTEC 6A blood culture vials (Becton Dickinson) containing soybean-casein digest broth. To account for killing of the exposed organisms, two further dilutions of the unexposed culture were made (final dilutions, 10^{-2}, 10^{-3}, and 10^{-4}) and the dilution closest to the exposed culture was selected as the control. In at least one experimental run of each antimicrobial agent-microorganism combination studied, an additional drug control was employed by inoculating similarly diluted unexposed organisms

* Corresponding author.

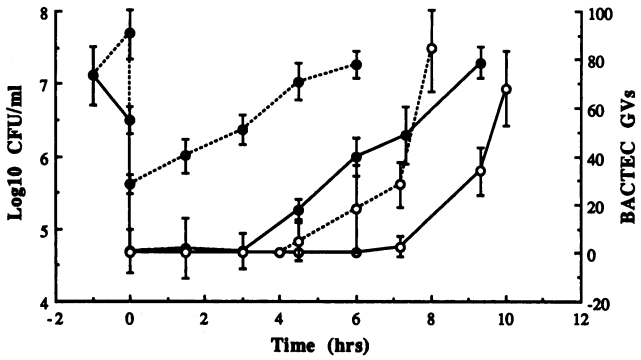

FIG. 1. Mean regrowth curves of exposed and unexposed control organisms (viability counting and BACTEC GVs) from five experiments involving *S. aureus* B5663 after 1 h of exposure to rifampin at a concentration of four times the MIC. Closed symbols, viability counts (log_{10} CFU per milliliter); open symbols, CO_2 generation (GV); interrupted lines, unexposed controls; continuous lines, exposed organisms. Error bars denote SDs.

into a fresh vial into which a 10^{-2} dilution of the highest drug concentration had been made. A drug control growing at the same rate as the unexposed control ensured that the PAEs observed were not due to residual subinhibitory levels of the drug (3).

The BACTEC vials were incubated on a shaker at 35.5°C. At 1- to 2-h intervals, CO_2 production in the vials was measured by using the BACTEC NR 730 system. Immediately thereafter, 100- to 200-μl samples were obtained from the same vials and viable counts were determined by plating serial 10-fold dilutions of the samples on MH agar and incubating them for 18 to 24 h at 35.5°C.

The PAE was calculated by the equation PAE = $T - C$, where for the BACTEC system, T is the time required for cumulative CO_2 production in exposed organisms to reach a GV of 30 and C is the time required for the untreated control culture to reach a cumulative GV of 30. By the viability method, T is the time required for the CFU count in the test culture to increase 1 log_{10} above the count observed immediately after drug removal and C is the time required for the CFU count in an untreated control culture to increase by 1 log_{10} above the count observed immediately after the identical removal procedure. The duration of the PAE was also calculated in most of the experiments by using lower cutoff GVs of 10, 15, and 20.

All experiments were repeated two to five times in duplicate on different days. Replicate test results were averaged. Outlying results were handled by the criterion of Dixon (14). The durations of the PAEs for individual microorganism-antimicrobial agent combinations determined by the two different methods were compared by paired Student t test. Correlation of the PAEs by each method was determined by least-squares linear regression. The slope of the regression line was compared with a line with a slope of 1 by the t test, and residuals from the regression line were evaluated by chi-square test.

As an example of typical experimental curves, Fig. 1 demonstrates average regrowth (viability counting and BACTEC GVs) from five experiments involving *S. aureus* B5663 after 1 h of exposure to rifampin at a concentration four times the MIC. As shown, logarithmic growth was well under way, even at a GV of 10.

In Fig. 2, the durations of the PAEs for the two *S. aureus* strains determined by the CO_2 generation (BACTEC) method and the standard viability count method are compared. No significant difference between the methods for each individual drug and concentration was observed. The mean differences in the durations of PAEs determined by the two methods were -0.1 ± 0.5 h for *S. aureus* ATCC 25923 and -0.1 ± 0.6 h for the clinical strain. For both strains, the combined mean difference was -0.1 ± 0.5 h (no significant difference).

Similarly, Fig. 3 demonstrates the durations of PAEs for the two strains of *E. coli* determined by the two different

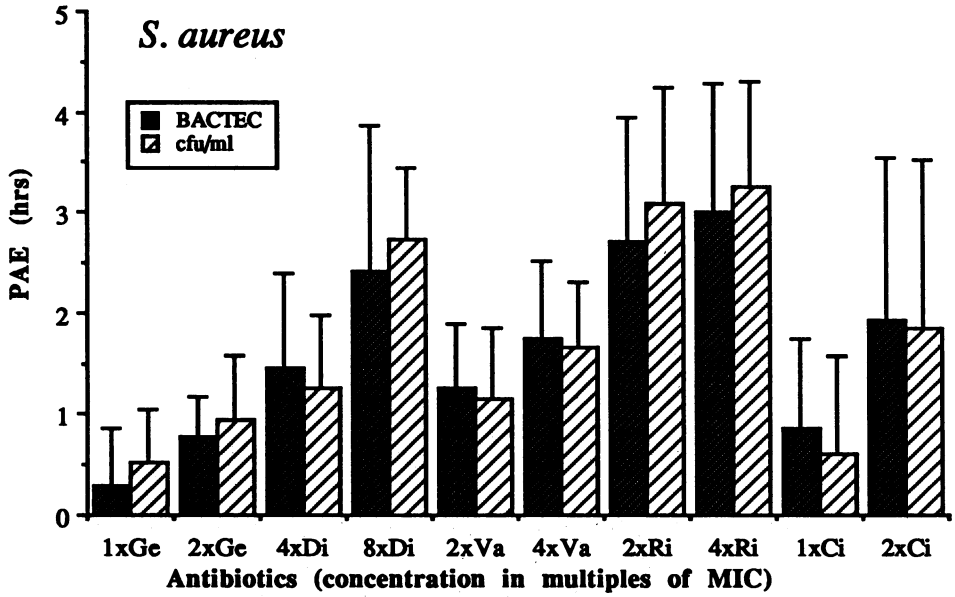

FIG. 2. Combined durations of the PAEs for *S. aureus* ATCC 25923 and B5663 as determined by the two different methods. Dark columns, mean PAE by CO_2 generation; light columns, mean PAE by viable counts. Bars denote SDs. Abbreviations: ampi, ampicillin; genta, gentamicin; cipro, ciprofloxacin.

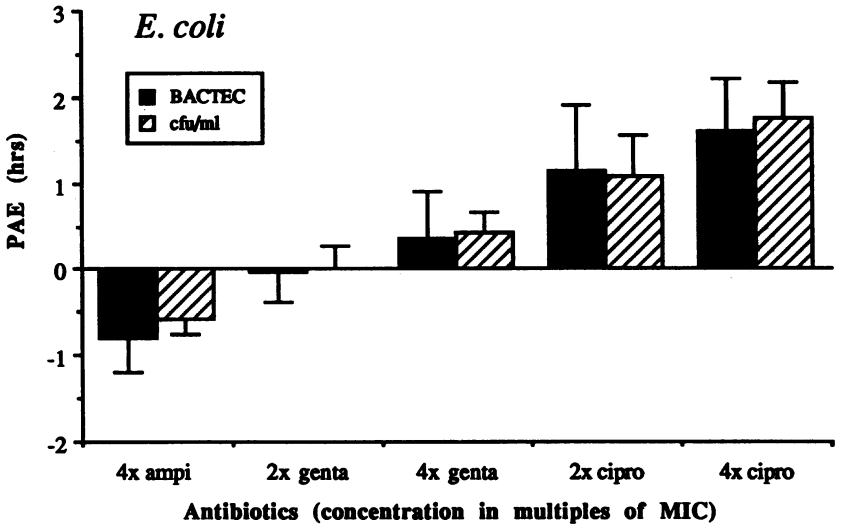

FIG. 3. Combined durations of PAEs for *E. coli* ATCC 25922 and B5756 as determined by the two different methods. Dark columns, mean PAE by CO_2 generation; light columns, mean PAE by viable counts. Bars denote SDs. Abbreviations: Ge, gentamicin; Di, dicloxacillin; Va, vancomycin; Ri, rifampin; Ci, ciprofloxacin.

methods, with no difference for individual drugs and concentrations demonstrated. The mean differences in the durations of PAEs by the two methods were -0.1 ± 0.3 h for *E. coli* ATCC 25922 and 0.0 ± 0.4 h for the clinical strain. For both strains combined, the mean difference was -0.1 ± 0.4 h (no significant difference).

The correlation coefficients of PAEs determined by the two different methods for *S. aureus* ATCC 25923 and B5663 were $r = 0.92$ and $r = 0.91$, respectively, and for both strains combined it was $r = 0.91$ ($P < 0.05$). For *E. coli* ATCC 25922 and B5756, the corresponding values were $r = 0.94$, $r = 0.91$, and $r = 0.94$, respectively ($P < 0.05$). For both species combined, the correlation coefficient was $r = 0.93$. The regression line had an intercept of 0.19 and a slope of 0.91 ± 0.08 (95% confidence intervals), which was not statistically different from a slope of 1.0. If the two methods were equivalent in determining the PAE, the slope would be 1.0 with an intercept of 0. The residuals about the regression line had a normal distribution without systematic deviation from the line, indicating that the two methods had equal variations.

GV of 30 was reached at viability counts of 7.2 ± 0.1 $\log_{10}$ CFU/ml (mean ± standard deviations [SD]) for *S. aureus* and 8.3 ± 0.2 $\log_{10}$ CFU/ml (mean ± SD) for *E. coli*, by which time the organisms had grown 1.5 to 2.5 $\log_{10}$ CFU/ml from the starting inoculum. Lower GV cutoffs of 10, 15, and 20 were reached for *S. aureus* at viability counts of 6.8 ± 0.2, 6.9 ± 0.1, and 7.1 ± 0.1 $\log_{10}$ CFU/ml (mean ± SD), respectively, and for *E. coli* at 7.6 ± 0.2, 7.8 ± 0.2, and 8.0 ± 0.2 $\log_{10}$ CFU/ml, respectively. GVs of 10 and 15 were reached ~1.0 h earlier than a GV of 30 both for the control and regrowth curves. Using the lower cutoff points thus did not affect the duration of the PAE perceptibly, with a difference between PAEs determined at GVs of 10 and 30 being 0.1 ± 0.1 h (mean ± SD).

By these observations, the correlation between the durations of the PAEs determined by the standard method of viability counting and by CO_2 generation in the BACTEC blood culture system is highly satisfactory.

For each experiment involving a single antimicrobial agent-organism combination, only three or four vials need to be employed. A single viability count needs to be performed on both the exposed and control cultures at the time of drug removal. At least two or three serially diluted control cultures need to be run, since the postdilutional (post-drug removal) inoculum of the control selected for calculation of the PAE needs to be close to the postdilutional inoculum of the exposed culture. Otherwise, underestimation of the bactericidal activity may result in overestimation of the PAE compared with viable counting. This may explain the discrepancy observed between PAEs determined by standard methods and PAEs determined by several alternate methods, such as optical density (2), electronic particle counting (9), and measurement of intracellular ATP content by bioluminescence assay (8, 11). In our studies, each 10-fold dilution of control cultures prolonged the time to reach a GV of 30 by 1.4 to 1.8 h for *S. aureus* and 1.1 to 1.2 h for *E. coli*.

Several antimicrobial agent-organism combinations can be investigated simultaneously without difficulty by the CO_2 generation method, and the total experimenter time required for the simultaneous run of five combinations is approximately 3 h. The PAEs of anaerobic organisms have not been studied in this system, but the anaerobic BACTEC 7D vials should be very suitable for such studies, although comparison with a standard anaerobic environment is obviously needed.

The major drawback of the method is that the experiment may frequently extend into the evening hours, particularly with drugs that induce long PAEs or exhibit rapid bactericidal activity. Measurable CO_2 generation may not be detected until 8 to 10 h after drug removal. By employing a GV cutoff lower than 30, this time may be shortened, but only by ~1.0 h. For the duration of the experiment, the BACTEC machine needs periodic, albeit short, attention, since the vials need to be placed manually into the sampling port of the BACTEC machine at the predetermined sampling times.

In conclusion, CO_2 generation as measured by the BACTEC blood culture system provides a simple, relatively inexpensive way of determining the PAE by using standard equipment present in many clinical microbiology laboratories.

This work was supported by grants from the Icelandic Science Foundation, the University of Iceland Research Fund, and the Reykjavik City Hospital Science Fund (S. Gudmundsson).

We thank the Becton Dickinson Company for supplying the blood culture vials.

REFERENCES

1. **Baquero, F., E. Culebras, C. Patrón, J. C. Pérez-Díaz, J. C. Moreno, and M. F. Vicente.** 1986. Postantibiotic effect of imipenem on Gram-negative and Gram-positive micro-organisms. J. Antimicrob. Chemother. **18**(Suppl. E):47–59.
2. **Bergan, T., I. B. Carlsen, and J. E. Fuglesang.** 1980. An in vitro model for monitoring bacterial responses to antibiotic agents under simulated in vivo conditions. Infection **8**:S96–S102.
3. **Craig, W. A., and S. Gudmundsson.** The postantibiotic effect, p. 403–431. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore.
4. **Goldenbaum, P. E. (Becton Dickinson and Co.).** Personal communication.
5. **Gould, I. M., A. C. Jason, and K. Milne.** 1989. Use of the Malthus microbial growth analyzer to study the post antibiotic effect of antibiotics. J. Antimicrob. Chemother. **24**:523–531.
6. **Hanberger, H., L. E. Nilsson, E. Kihlström, and R. Maller.** 1990. Postantibiotic effect of β-lactam antibiotics on *Escherichia coli* evaluated by bioluminescence assay of bacterial ATP. Antimicrob. Agents Chemother. **34**:102–106.
7. **Isakson, B., L. Nilsson, R. Maller, and L. Sörén.** 1988. Postantibiotic effect of aminoglycosides on gram-negative bacteria evaluated by a new method. J. Antimicrob. Chemother. **22**:23–33.
8. **Mattie, H.** 1981. Kinetics of antimicrobial action. Rev. Infect. Dis. **3**:19–27.
9. **Nadler, H. L., W. A. Curby, P. Forgacs, and F. Rosenberg.** 1989. Comparison of electronic and viability counting methods for determination of the postantibiotic effect of oxacillin on *Staphylococcus aureus*. Antimicrob. Agents Chemother. **33**:2155–2156.
10. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Odenholt, I., B. Isaksson, L. Nilsson, and O. Cars.** 1989. Postantibiotic and bactericidal effect of imipenem against *Pseudomonas aeruginosa*. Eur. J. Clin. Microbiol. Infect. Dis. **8**:136–141.
12. **Rescott, D. L., D. E. Nix, P. Holden, and J. J. Schentag.** 1988. Comparison of two methods for determining in vitro postantibiotic effects of three antibiotics on *Escherichia coli*. Antimicrob. Agents Chemother. **32**:450–453.
13. **Shah, P. M., K.-G. Huebner, and W. Stille.** 1978. In-vitro-untersuchungen zur intermittierenden Therapie mit Penicillin G und Ampicillin. Med. Welt **29**:888–892.
14. **Snedecor, G. W., and W. G. Cochran.** 1980. Statistical methods, 7th ed., p. 280. The Iowa State University Press, Ames.
15. **Sturm, A. W.** 1989. Netilmicin in the treatment of gram-negative bacteremia: single daily versus multiple daily dosage. J. Infect. Dis. **159**:931–937.
16. **Tulkens, P. M., F. Clerck-Braun, J. Donnez, S. Ibrahim, Z. Kallay, M. Delmee, P. Jacqmin, M. Gersdorff, M. Lesne, L. Kaufman, and M. P. Derde.** 1988. Safety and efficacy of aminoglycosides once-a-day: experimental data and randomized, controlled evaluation in patients suffering from pelvic inflammatory disease. J. Drug Dev. **1**(Suppl. 3):71–82.
17. **Winstanley, T. G., and J. G. M. Hastings.** 1989. Penicillin-aminoglycoside synergy and post-antibiotic effect for enterococci. J. Antimicrob. Chemother. **23**:189–199.