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Pharmacokinetic Determinants of Penicillin Cure of Gonococcal Urethritis

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In a 1964 study of the pharmacokinetic determinants of penicillin cure of gonococcal urethritis, 45 male prisoner volunteers were experimentally infected with strains of *Neisseria gonorrhoeae* having known in vitro penicillin susceptibility. After developing urethritis, subjects received intramuscular penicillin G and had serum samples obtained serially to determine penicillin concentration. Using a multiple regression technique, we studied patient-associated parameters and parameters of the serum penicillin curves to determine the best predictors of treatment results. Cure was best predicted by the time the serum penicillin concentration remained above three to four times the penicillin minimum inhibitory concentration of the infecting strain (probability of correct classification, >0.80). Those cured had serum penicillin concentrations which remained in this range for means of 7 to 10 h. Our findings confirm principles of antimicrobial therapy derived from animal models and may have application in studying therapy of gonorrhea and other infectious diseases.

Although the initial use of penicillin provided both simple and effective therapy for gonorrhea, by the early 1960s therapeutic problems had developed in the United States. The in vitro penicillin resistance of gonococcal isolates had increased (15). Commonly used penicillin regimens had failure rates estimated to be as high as 50% (14).

To provide a rational basis for new treatment regimens, James Thayer and associates began a study in human volunteers to examine the relationship of penicillin cure of gonorrhea to serum penicillin levels and in vitro resistance of gonococcal isolates. Although the study was done in 1964, data analysis was never completed because of Dr. Thayer's death. In this paper we reanalyze the study data by using modern statistical techniques.

MATERIALS AND METHODS

Study design and population. Between March and June 1964, 47 male inmates of the United States Penitentiary, Atlanta, Ga., entered the study with written informed consent. All subjects were over age 21 years, in good health, and had not had gonorrhea within the past 3 months. They all denied penicillin allergy and had negative intradermal skin tests with benzylpenicilloyl-polylysine antigen. For all subjects, culture specimens taken from the urethra, rectum, nasopharynx, oropharynx, and urine sediment, both before and after prostatic massage, showed no growth of *Neisseria gonorrhoeae* on GC agar base containing 1% of the defined supplement described by White and Kellogg (19) and on Thayer-Martin medium (16).

After admission to a prison hospital isolation ward, subjects received an intraurethral inoculation with a 2-mm platinum loop containing approximately 15×10^9 colony-forming units of *N. gonorrhoeae*. For each subject the inoculum was taken from a culture plate having an 18- to 24-h growth of a gonococcal strain previously isolated from a venereal disease clinic patient. At least 95% of the colonies on these plates were type 1 (11). Three different strains were used in the study. By using a plate dilution method (17), these strains had been shown to have penicillin minimal inhibitory concentrations (MICs) of 0.25, 0.30, and 1.20 U/ml.

A total of 45 subjects developed a purulent urethral discharge which was culture positive for *N. gonorrhoeae*. Gonococcal identity was confirmed in all cases with sugar utilization testing. At 2 days after the development of symptomatic urethritis, subjects were treated intramuscularly with penicillin according to the following schedules: (i) 0.9×10^6 U of procaine penicillin G in oil with 2% aluminum monosterate (PAM) (8 men); (ii) 1.2×10^6 U of PAM (7 men); (iii) 2.4×10^6 U of PAM (7 men); (iv) 1.2×10^6 U of aqueous procaine penicillin G (APPG) (8 men); (v) 2.4×10^6 U of APPG (8 men); and (vi) 1.0×10^6 U of

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aqueous potassium penicillin G followed by 0.4×10^6 U 3 h later (7 men).

After treatment, serum specimens were serially obtained at 1, 2, 4, 6, 12, 18, 24, 36, 48, and 72 h for groups (i) through (v) and at 1, 2, 3, 4, 8, 10, 12, 16, and 20 h for group (vi). These sera were tested for penicillin concentration by a *Sarcina lutea* cup assay (7). Urethral and urine sediment specimens, serially obtained for 96 h, were cultured on GC agar base and on Thayer-Martin medium.

Subjects were considered to be cured if culture specimens taken at 72 and 96 h after treatment were negative for *N. gonorrhoeae*. Those not cured were retreated either with oxytetracycline (500 mg given orally as an initial dose, followed by 250 mg every 6 h for 48 h) or with 4.0×10^6 to 5.0×10^6 U of APPG given intramuscularly. After cure, subjects were released from the isolation ward, but were reexamined weekly for 2 weeks and then monthly for a total follow-up of 3 months. At the conclusion of the study all subjects were cured; none developed any complications of gonococcal infection or had any adverse reactions to treatment drugs.

Statistical technique. To examine the relationship of serum penicillin levels to treatment results, it was necessary to find equations to describe the serum penicillin curve for each study subject. Initially we attempted to fit each curve to a one-compartment open model by using the method described by Metzler et al. (13). Although this model gave a good fit for the 20 subjects whose serum penicillin curves had a single peak, we were unable to fit the double-peak curves of the other 25 participants. To overcome this difficulty, we used another method, the grafted polynomial of degree k (6), to fit the curves of all 45 subjects. This method, which is used when a function is believed to be a "smooth" function of time but the function form is not known, involves approximating segments of the function by low-order polynomials and then joining the segments to form a continuous function.

Using the grafted polynomial method, we then calculated a number of parameters of each serum curve. Examples of these parameters include: total area under the curve, time to peak, area of the curve above the MIC of the infecting strain, ratio of area above the MIC to total area under the curve, ratio of area above the MIC to area below the MIC, time above the MIC, and differences between peak serum penicillin level and MIC. For all parameters which included MIC, we also created additional parameters with multiples of the MIC, e.g., time the curve remained above four times the MIC. These curve variables plus subject variables (age, race, height, weight, and surface area) were then studied by discriminant analysis, a multivariate technique which allowed us to test for differences between subjects who were cured and subjects who were not cured. In all, 46 variables were studied to determine which variables were most likely to correctly classify subjects as treatment cures or failures.

There are several methods for identifying the significant variables in discriminant analysis. In the stepwise method we used (2), the variable having the greatest discriminating power enters first into the analysis. The second variable to enter is the variable that produces the greatest increase in discriminating power, given that the first variable has already entered. Variables continue to enter based on their increase of the discriminating power produced by the previously entered variables. At each step of the analysis, one can test if the increase in discriminating power attributed to a new variable is statistically significant.

RESULTS

Complete demographic information was available for 44 of the 45 study subjects. Of these 44 men, 37 were white and 7 were black. Their ages ranged from 22 to 47 years (mean, 35.6 years).

A total of 23 men were cured with their initial penicillin therapy, and 22 were not cured. Of those who were cured, 52.2% had negative urethral specimen cultures at 6 h after their first penicillin injection, and all had negative cultures by 36 h. Of those who were not cured, 18.2% had transiently negative urethral specimen cultures, but all had positive cultures when tested at 72 h after treatment.

Figure 1 shows the curves of mean serum penicillin levels for subjects cured and not cured by each penicillin regimen. The PAM regimens tended to produce relatively low peak levels, followed by a sustained, low-level penicillinemia. In contrast, the APPG and aqueous penicillin regimens produced higher peak serum levels, but penicillinemia was less sustained than with PAM. For the aqueous penicillin regimen, a second concentration peak clearly followed the penicillin injection given 3 h after initial therapy. Several of the PAM curves also show small secondary peaks at 24 h, although these occurred in the absence of further therapy.

In all treatment regimens, serum penicillin levels were higher for those who were cured than for those who were not cured for at least 6 h after therapy. Concentration differences as high as 10-fold occurred among individuals cured and not cured by the same regimens.

Table 1 lists 10 representative parameters of the serum penicillin concentration curve in the order by which they could correctly classify subjects as treatment cures or failures. From this analysis, a major determinant of cure appears to be the time the serum penicillin concentration remains above three to four times the penicillin MIC of the infecting strain. Those cured had serum penicillin concentrations which remained in this range for means of 7 to 10 h versus 2 or less for the failures. Variables representing ratios of the curve area above multiples of the MIC to total area under the curve were also good predictors of treatment results.

Many of the variables used in the analysis were highly correlated with each other. For example, two variables which were good individual

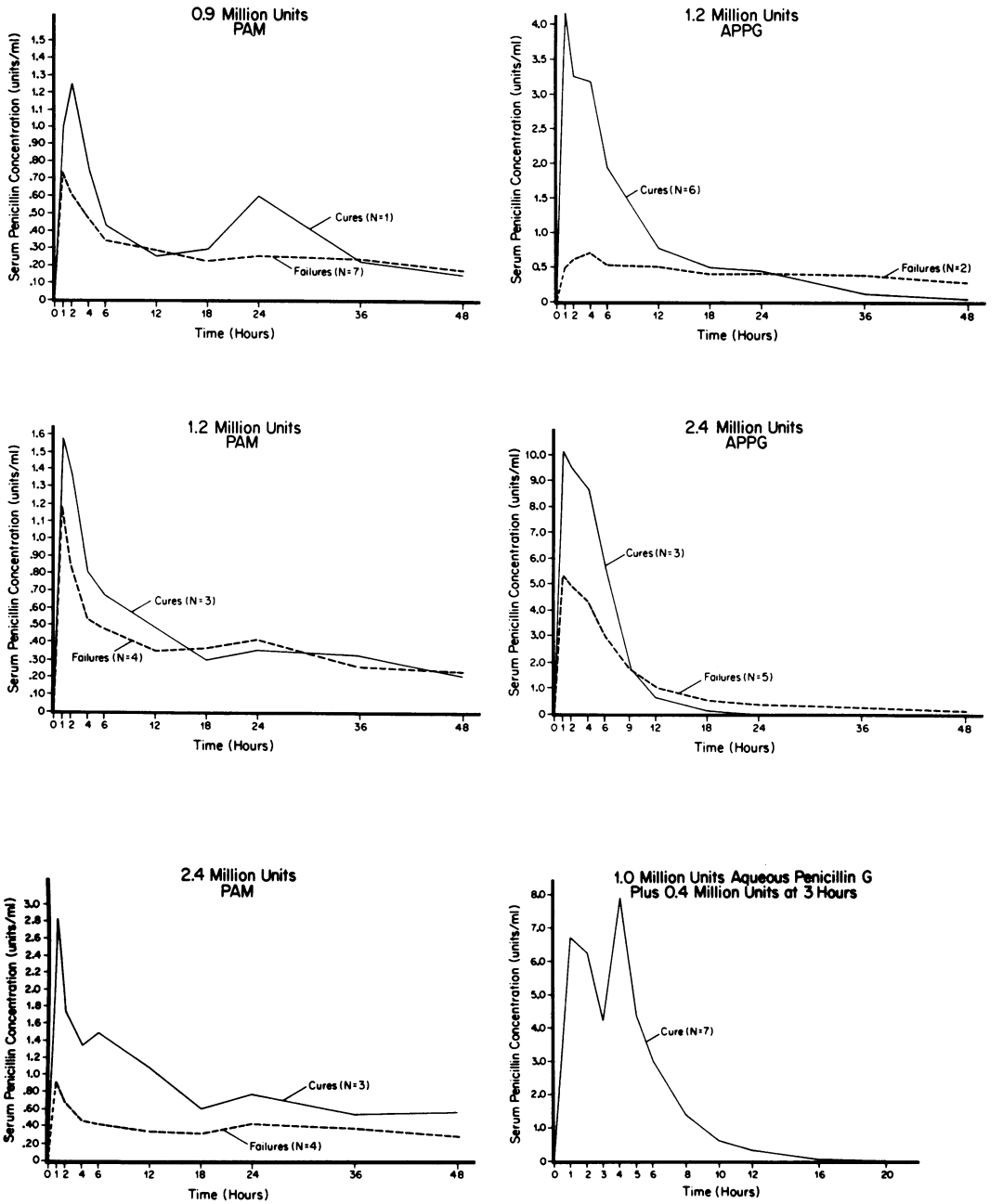

FIG. 1. *Mean serum penicillin concentrations for subjects cured and not cured by each penicillin regimen.*

predictors of treatment result, duration of penicillinemia greater than four times the MIC and area above the MIC divided by total area under the curve, had a correlation coefficient of 0.71. Because of this sort of correlation, once variables relating to time the penicillin concentration remains above multiples of the MIC enter into the stepwise discriminant analysis, no other variables significantly increase the discriminating power of the analysis.

Patient-associated variables did not appear to be important predictors of treatment results. None of these variables (race, age, height, weight, and surface area) had a probability of

TABLE 1. *Parameters of the serum penicillin concentration curve as predictors of treatment result*

Parameter	Probability of correction classification	Mean of parameter for:			
		Cures		Failures	
Time above 4× MIC	0.85	6.9 h	(3.8)[a]	0.78 h	(1.4)
Area above MIC/total area	0.84	0.72	(0.24)	0.26	(0.22)
Time above 3× MIC	0.81	9.9 h	(5.8)	2.0 h	(2.4)
Area above 4× MIC	0.79	15.4	(11.1)	1.7	(4.1)
Difference between peak and 4× MIC	0.78	4.6 U/ml	(3.7)	0.10 U/ml	(1.9)
Area above MIC	0.76	34.0	(19.9)	10.1	(13.5)
Peak concentration	0.72	6.2 U/ml	(4.1)	2.0 U/ml	(2.8)
Time above 2× MIC	0.69	15.4	(14.8)	4.6	(4.7)
Total area under curve	0.67	45.6	(20.1)	29.2	(18.0)
Time above MIC	0.53	30.0 h	(21.7)	26.6 h	(27.3)

[a] Numbers in parentheses are standard deviations.

correct classification of more than 0.59. Furthermore, there was no statistically significant relationship between any of these patient-associated variables and the peak serum concentration or total area under the curve for a particular penicillin regimen.

DISCUSSION

Soon after the introduction of penicillin, Eagle and associates began investigations of the relationships between time and dose of penicillin therapy and cure of experimental infection (3–5). In a series of studies on streptococcal, pneumococcal, and treponemal infections in mice and rabbits, these workers found that the critical determinant of cure was the time that the serum penicillin concentration remained above a certain concentration, regardless of the method of penicillin administration. This optimal concentration, a multiple of the concentration required to inhibit microbial growth in vitro varied with the species of the infecting organism and the site of infection. Cure could occur with suboptimal penicillin concentrations, but the time of treatment was prolonged. Penicillin concentrations higher than the optimal concentration did not speed recovery.

Despite the precedent of these elegant studies in animals, there followed little analogous work in human infections. New treatment recommendations for gonorrhea have been largely based on clinical trials of antibiotic regimens, without a detailed understanding of the optimal serum antibiotic curve. The few studies which have examined the relationship of serum antibiotic levels to treatment results suffer from methodological problems. In a 1965 study of 16 patients, Krook and Juhlin found cure rates to be highest when peak serum penicillin levels were 5 to 10 times the penicillin MIC of the infecting gonococcal strain (12). More recently Kandhari et al. suggested that for gonococcal urethritis optimal penicillin treatment produces serum bactericidal levels of 1:128 within 2 h after treatment, which should be maintained for at least 8 h (9). However, neither study examined more than a few arbitrarily selected parameters of the serum penicillin curve, and in both studies reinfection may have caused patients to be falsely classified as treatment failures.

We believe that Dr. Thayer's study avoids these methodological problems and demonstrates the applicability of the principles of Eagle et al. to gonococcal infection in humans. Cure of gonococcal urethritis in men is most likely to occur when serum penicillin levels reach three to four times the MIC of the infecting strain and remain at these levels for approximately 7 to 10 h. Cure is also most likely when the ratio of curve area above multiples of the MIC to the total area under the curve is high. This ratio is strongly correlated with duration of penicillinemia at three to four times the MIC and may reflect the shape of the curve which is needed to produce the optimal time-concentration relationship.

In this study, wide variation of serum penicillin concentration occurred among individuals receiving identical penicillin regimens. Other investigators have reported similar variations after intramuscular penicillin therapy (18, 20). We could not relate the observed variation to race, age, or size of study subjects. Although renal function was not assessed, none of the subjects was known to have chronic renal disease. Presumably these variations in penicillin concentration reflect individual differences in rate of penicillin absorption, volume of distribution, and rate of elimination.

The small second peak in serum penicillin concentration which occurred at 24 h after PAM injection has not been described in other studies of PAM. Since subjects were treated in the morning, the 24-h serum specimen was drawn

after subjects were awake and active the following morning. We believe that the 24-h peak may reflect increased release of penicillin from muscle, secondary to increased physical activity, rather than some intrinsic property of the PAM preparation.

We have no direct way to relate the findings of this 1964 study to the current treatment of gonorrhea with the United States Public Health Service recommended regimen of 4.8×10^6 U of APPG plus 1.0 g of probenecid given orally. We believe, however, that the pharmacokinetic relationships found in this study should still apply today. The gonococcal strains which were used in this study have penicillin MICs which are within the range of susceptibility encountered in recent years (8). However, in a study of eight male volunteers, Adams et al. found that the currant APPG-probenecid regimen produces peak serum penicillin concentrations of 28 to 57 U/ml (17 to 34 μg/ml) and maintains concentrations above a mean of 10 U/ml for more than 12 h (1). These levels are considerably higher than those obtained with any of the regimens used in this study. Since the current regimen would be very likely to produce and maintain serum penicillin concentrations in excess of three to four times the MIC of an infecting strain, it is not surprising that the current regimen has a cure rate of 97.3% for gonococcal urethritis (10).

Since none of the parameters of the serum penicillin curve was a perfect predictor of treatment result, other factors must also contribute to the result. Clearly, the serum curve is only an indicator rather than a direct measurement of penicillin concentration at the infection site. Penicillin concentration in other body fluids, such as urine and prostatic secretions, may also be important in the treatment of gonococcal urethritis. Furthermore, host defense mechanisms may contribute to the cure achieved by antibiotic therapy.

The data presented here may provide a rational basis for formulation of future penicillin regimens for the treatment of gonococcal urethritis. The type of analysis used in this study may also have application in the study of antimicrobial therapy of other infectious diseases.

LITERATURE CITED

1. **Adams, H. G., M. Turck, and K. K. Holmes.** 1975. Comparison of aqueous sodium penicillin G in lidocaine and aqueous procaine penicillin G for the treatment of gonorrhea, p. 279–284. *In* D. Danielsson, L. Juhlin, and P. A. Mardh (ed.), Genital infections and their complications. Almqvist and Wiksell International, Stockholm.
2. **Dixon, W. J.** 1974. BMD, biomedical computer programs, p. 233–245. University of California Press, Los Angeles.
3. **Eagle, H., R. Fleischman, and M. Levy.** 1953. "Continuous" vs. "discontinuous" therapy with penicillin. The effect of the interval between infections on therapeutic efficacy. N. Engl. J. Med. **248**:481–488.
4. **Eagle, H., R. Fleischman, and A. D. Musselman.** 1950. Effect of schedule of administration on the therapeutic efficacy of penicillin. Importance of the aggregate time penicillin remains at effectively bactericidal levels. Am. J. Med. **9**:280–299.
5. **Eagle, H., R. Fleischman, and A. D. Musselman.** 1950. The effective concentrations of penicillin in vitro and in vivo for streptococci, pneumococci, and *Treponema pallidum*. J. Bacteriol. **59**:625–643.
6. **Fuller, W. A.** 1976. Introduction to statistical time series, p. 393–397. John Wiley & Sons, Inc., New York.
7. **Grove, D. C., and W. A. Randall.** 1955. Assay methods of antibiotics. A laboratory manual, p. 14–16. Medical Encyclopedia, Inc., New York.
8. **Jaffe, H. W., J. W. Biddle, C. Thornsberry, R. E. Johnson, R. E. Kaufman, G. H. Reynolds, P. J. Wiesner, and the Cooperative Study Group.** 1976. National gonorrhea therapy monitoring study. In vitro antibiotic susceptibility and its correlation with treatment results. N. Engl. J. Med. **294**:5–9.
9. **Kandhari, K. C., J. S. Pasricha, N. C. Bhargava, A. K. Sharma, O. P. Singh, P. Sood, and R. A. Bhujwala.** 1975. Penicillic therapy in gonorrhea: correlation of in vitro resistance of *N. gonorrhoeae* strains with clinical response to penicillin and its blood levels. Indian J. Med. Res. **63**:818–823.
10. **Kaufman, R. E., R. E. Johnson, H. W. Jaffe, C. Thornsberry, G. H. Reynolds, P. J. Wiesner, and the Cooperative Study Group.** 1976. National gonorrhea therapy monitoring study. Treatment results. N. Engl. J. Med. **294**:1–4.
11. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274–1279.
12. **Krook, G., and I. Juhlin.** 1965. Problems in diagnosis, treatment and control of gonorrheal infections. IV. The correlations between the dose of penicillin, concentration in blood, IC_{50}-values of gonococci and results of treatment. Acta Derm. Venereol. **45**:242–253.
13. **Metzler, C. M., G. L. Elfring, and A. J. McEwen.** 1974. A users manual for nonlinear and associated programs. The Upjohn Co., Kalamazoo, Mich.
14. **Simpson, W. G., and W. J. Brown.** 1962. Current status of the diagnosis and management of gonorrhea. J. Am. Med. Assoc. **182**:63–66.
15. **Thayer, J. D., F. W. Field, M. I. Perry, J. E. Martin, Jr., and W. Garson.** 1961. Surveillance studies of *Neisseria gonorrhoeae*. Sensitivity to penicillin and nine other antibiotics. Bull. W.H.O. **24**:327–331.
16. **Thayer, J. D., and J. E. Martin, Jr.** 1964. A selective medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. Public Health Rep. **79**:49–57.
17. **United States Public Health Service.** 1960. Gonococcus—procedures for isolation and identification. Publication no. 499, p. 23–28. U.S. Government Printing Office, Washington, D.C.
18. **White, A. C., R. A. Couch, F. Foster, J. Calloway, W. Hunter, and V. Knight.** 1956. Absorption and antimicrobial activity of penicillin V (phenoxymethyl penicillin). Antibiot. Annu. **1955–1956**:490–501.
19. **White, L. A., and D. S. Kellogg, Jr.** 1965. *Neisseria gonorrhoeae* identification in direct smears by a fluorescent antibody-counterstain method. Appl. Microbiol. **13**:171–174.
20. **Whittlesey, P., and W. L. Hewitt.** 1948. Serum concentrations of penicillin following administration of crystalline procaine penicillin G in aqueous suspension. Proc. Soc. Exp. Biol. Med. **68**:658–661.