ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1977, p. 479-483

Vol. 12, No. 4
Printed in U.S.A.

Antibiotic Concentrations in Serum, Serum Bactericidal Activity, and Results of Therapy of Streptococcal Endocarditis in Rabbits

JAIME CARRIZOSA AND DONALD KAYE*

Department of Medicine, The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

Received for publication 10 May 1977

The correlation between antibiotic concentrations in serum, serum bactericidal activity, and results of therapy was studied in rabbits with streptococcal endocarditis. Five days of procaine penicillin G reduced bacterial titers to <10 per g in 12 of 14 vegetations in rabbits receiving 75,000 U intramuscularly every 6 h and 10 of 20 in rabbits given 37,500 U. Ten days of 18,750 U every 6 h did not reduce the titers. To test for cure, rabbits were treated with 75,000 U every 6 h for 10 or 20 days and then received no therapy for 7 days. At the end of the 7-day period without therapy, vegetations were sterile in five of five and eight of eight animals, respectively. Rabbits received 37,500 U every 6 h for 5, 10, or 20 days and then no therapy for 7 days, after which vegetations were sterile in one of seven, four of nine, and seven of eight animals, respectively. The median maximal serum bactericidal dilutions at 1 h were 1/16 when 75,000 U of procaine penicillin G was administered, 1/8 to 1/16 with 37,500 U, and 1/4 to 1/8 with 18,750 U. Serum bactericidal activity could not be detected in 50% of the rabbits 6 h after administration of 37,500 U. Cure was related to a median maximal serum bactericidal dilution of at least 1/8 to 1/16 1 h after penicillin administration. A median maximal serum bactericidal dilution of 1/4 to 1/8 resulted in unsuccessful therapy.

Appropriate treatment of bacterial endocarditis is based on accurate identification of the microorganism, antibiotic susceptibility patterns determined by a broth dilution technique to establish minimal inhibitory and bactericidal concentrations, and determinations of the patient's serum bactericidal activity against the causative microorganism. Although there are no controlled studies, the clinical impression has been that for successful antibiotic treatment the patient's serum at the time of peak activity should be bactericidal for the infecting organism at a dilution of at least 1:8 (1, 2, 7, 9).

The present study was designed to correlate the results of stretptococcal endocarditis treatment in rabbits with serum antibiotic concentrations, peak serum bactericidal activity, and duration of therapy.

MATERIALS AND METHODS

Microorganism. A strain of *Streptococcus viridans* subspecies *mitis* isolated from a patient with endocarditis was used in all experiments. The minimal inhibitory concentration and minimal bactericidal concentration (MBC) of penicillin G were both 0.1 μg/ml in rabbit serum and in heart infusion broth (HIB) (Difco Laboratories, Detroit, Mich.). These were determined by a twofold antibiotic dilution method in rabbit serum or HIB with 5% sheep erythrocytes, using an inoculum of 10^6 colony-forming units (CFU) of streptococci per ml. Stock cultures were made by storing portions of an 18-h culture in HIB with 5% sheep erythrocytes at −20°C. For each experiment, a 1-ml aliquot was thawed, inoculated into 50 ml of HIB with 5% sheep erythrocytes, and incubated for 18 h at 37°C.

Production of endocarditis. Female white New Zealand rabbits (West Jersey Biological Farm, Wenonah, N. J.) weighing 2 to 2.5 kg were anesthetized with sodium pentobarbital intravenously, and the right carotid artery was exposed. Polyethylene tubing was passed through the carotid artery into the left ventricle and was secured as previously described (3, 4). Twenty-four hours later, 10^8 CFU of streptococci in a 1-ml volume of HIB with 5% sheep erythrocytes was injected into a marginal ear vein. This procedure reproducibly causes endocarditis with vegetation on the aortic valve.

Treatment. Rabbits were treated with different concentrations of procaine penicillin G ranging from 4,688 to 300,000 U injected intramuscularly every 6 or every 12 h. Therapy was started 24 h after infection and continued for varying periods of time. Control rabbits were not treated.

Experiments. After varying intervals of therapy, following a period of at least 6 h without treatment, the rabbits were anesthetized by intravenous injection of sodium pentobarbital. Right atrial blood

specimens were obtained for blood cultures, and the aortic valve vegetation was excised and weighed. The vegetations weighed 0.03 to 0.4 g. After homogenizing a 1:10 suspension of each vegetation containing 1,000 U of penicillinase per ml in HIB, the number of CFU was determined by serial dilution and plating techniques as previously described (3). The number of CFU was expressed as $\log_{10}$ CFU per gram. In performing statistical analyses, sterile vegetations were counted as $\log_{10}$ 1 CFU/g, as bacteria were present in all vegetations weighing less than 0.1 g, and 0.1 g was plated in all of the vegetations without growth. Student's t test was used to determine significant differences.

Blood was periodically taken from ear veins for culture. Cultures of blood were made by plating 0.1- and 1-ml samples on the surfaces of blood agar plates and incubating at 37°C for 48 h. Blood was also removed at intervals after penicillin injection for antibiotic concentration and bactericidal activity determinations.

Penicillin concentrations in serum and serum bactericidal activity. After removing the serum, penicillin concentrations were assayed by an agar diffusion method with paper disks (10). Serum bactericidal activity was determined by a modification of the method of Barry and Sabath (1). The serum was diluted in twofold steps in tubes containing 0.5 ml of rabbit serum, and to this 0.5 ml of serum with 5% sheep erythrocytes containing 10^6 CFU of an 18-h HIB culture of the streptococcus was added. After incubation for 24 h at 37°C, 0.01 ml of the contents of each tube was plated on the surface of a blood agar plate. The maximal bactericidal dilution (MBD) was the highest dilution of serum that resulted in less than five colonies on the plate after 48 h of incubation at 37°C. This represents at least 99.9% kill (1).

RESULTS

Thirteen rabbits were injected intravenously with 10^8 CFU of streptococci, and the mortality rate was 15% on day 7 of infection. Seventeen similarly infected, untreated rabbits were killed on days 1, 3, 5, and 7 of infection. All had endocarditis with a mean $\log_{10}$ CFU count of 7.9 streptococci per g of vegetation or higher on each of the 4 days studied.

Rabbits were treated with 300,000, 150,000, 75,000, 37,500, 18,750, 9,375, or 4,688 U of procaine penicillin intramuscularly every 6 h. Vegetation titers were not decreased with 24 h of therapy (mean $\log_{10}$ CFU/g of vegetation was 8.5 to 8.9 for the treatment groups as compared with 8.3 for untreated controls). When treatment was extended to 3 days, only rabbits treated with 300,000 U every 6 h had decreased titers in the vegetations (mean $\log_{10}$ CFU ± standard deviation [SD] of 5.7 ± 1.2 per g in five rabbits as compared with 7.9 ± 0.8 for five untreated controls, $P < 0.05$).

Figure 1 shows the results in groups of rabbits treated for 5 days with 4,688 to 75,000 U of penicillin every 6 h. In animals receiving 75,000 U, 12 of 14 vegetations showed no growth (i.e., <10 CFU/g), and the remaining two rabbits had 2.0 and 2.2 $\log_{10}$ CFU/g of vegetation, respectively ($P < 0.001$ when compared with controls where vegetations contained 8.1 ± 0.6 mean $\log_{10}$ CFU/g). With 37,500 U of penicillin per dose, mean vegetation titers were 1.7 ± 1.0 CFU/g, and 10 of 20 vegetations had no growth ($P < 0.001$ when compared with controls). With the 18,750-U dose, all vegetations had growth. The mean titer of 5.6 ± 2.0 CFU/g was significantly lower than in controls ($P < 0.001$) and significantly higher than with 37,500 U per dose ($P < 0.001$). With 9,375 and 4,688 U per dose, titers were 7.0 ± 1.6 and 7.6 ± 1.1 CFU/g, respectively (not different when compared with controls [$P > 0.05$] but significantly higher than with 37,500 U per dose [$P < 0.001$]).

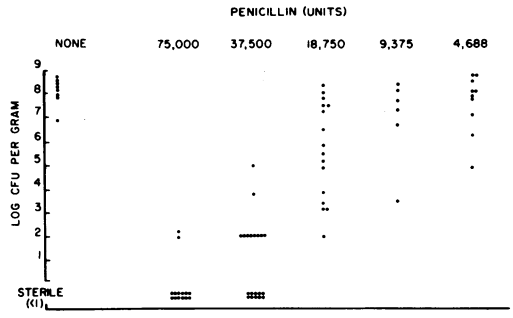

FIG. 1. *$\log_{10}$ CFU of streptococci in aortic valve vegetations of rabbits with endocarditis treated for 5 days with varying doses of penicillin given intramuscularly every 6 h; controls received no penicillin. Each dot represents one vegetation.*

Although 5 days of 37,500 U every 6 h was sufficient to result in vegetations containing <10 streptococci per g in 50% of rabbits, this regimen was not curative in these animals. When therapy with 37,500 U was discontinued after 5 days and rabbits were killed after 7 more days without treatment, only 1 of 7 had no growth from vegetations and the rest had 6.2 to 9.0 $\log_{10}$ CFU/g. Therefore, additional experiments were performed to evaluate the effect of a longer duration of therapy on curing endocarditis.

Rabbits were treated with 9,375, 18,750, 37,500, or 75,000 U of procaine penicillin every 6 h for 10 days. Ten days of treatment with 9,375 or 18,750 U was insufficient to reduce titers in vegetations. At the end of 10 days of therapy, animals treated with these doses had vegetations containing 8.5 ± 0.6 and 7.2 ± 1.4 mean $\log_{10}$ CFU/g, respectively ($P > 0.05$ as compared with untreated controls [8.4 ± 0.7]).

In contrast, at the end of 10 days of 75,000 U of penicillin every 6 h, vegetations from all 5 rabbits studied were negative for streptococci, and when animals were killed after another 7 days without penicillin, 5 of 5 had vegetations negative for bacteria. Therapy with 37,500 U of penicillin was not as effective. At the end of 10 days of treatment with 37,500 U, vegetations from 3 of 5 animals were negative for bacteria and the other 2 contained 1.7 and 2.9 $\log_{10}$ CFU/g; after 7 days without penicillin, only 4 of 9 had negative cultures of vegetations. (The other five had 5.2 mean $\log_{10}$ CFU/g of vegetation.) After 20 days of penicillin treatment and then 7 days without therapy, 8 of 8 rabbits given 75,000 U every 6 h and 7 of 8 rabbits given 37,500 U every 6 h had sterile vegetations.

Further studies were performed to determine the effect of decreasing the every-6-h injections to every-12-h injections. Rabbits were treated with 300,000, 150,000, 75,000, or 37,500 U of procaine penicillin G twice daily intramuscularly for 5 days (Fig. 2). Of these, 8 of 9 rabbits treated with 300,000 U showed no growth in the vegetations; the remaining rabbit had 2.0 $\log_{10}$ CFU/g of vegetation. With 150,000 U, the mean $\log_{10}$ CFU ± SD was 2.1 ± 1.1 per g and three of seven rabbits showed no growth in the vegetations. With 75,000 U, the mean $\log_{10}$ CFU ± SD was 4.5 ± 3.0, and four of eleven rabbits showed no growth. With 37,500 U, the mean titer was 6.8 ± 3.0 CFU/g, and only one animal showed no growth ($P < 0.001$ for all comparisons with controls, 8.3 ± 0.6 $\log_{10}$ CFU, except for the 37,500-U group, in which P was >0.05).

Blood cultures. One day of therapy with 300,000 U or smaller doses of procaine penicillin every 6 h was inadequate to sterilize blood cultures. After 24 h of treatment, 60% of rabbits receiving 300,000 U and a higher percentage of those treated with lower doses had positive blood cultures. Blood cultures obtained after 5 days of treatment with 300,000, 150,000, 75,000, or 37,500 U of procaine penicillin every 6 h were all sterile. However, even after 10 days of treatment with 18,750 U of penicillin every 6 h, 50% of the blood cultures were positive for streptococci.

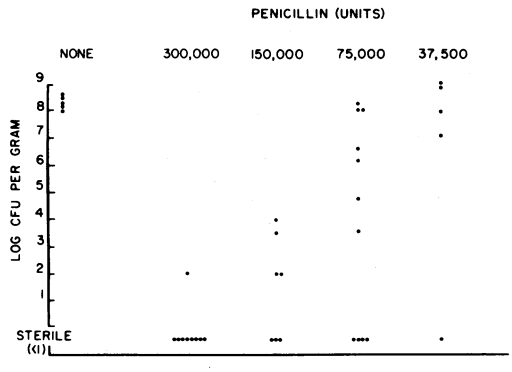

FIG. 2. *$\log_{10}$ CFU of streptococci in aortic valve vegetations of rabbits with endocarditis treated for 5 days with varying doses of penicillin given intramuscularly every 12 h; controls received no penicillin. Each dot represents one vegetation.*

Concentrations of penicillin and antibacterial activity in serum. Table 1 shows the mean and median penicillin levels in serum after the first dose of penicillin. There was no increase in serum concentrations of penicillin after an injection on day 5 of therapy as compared with Table 1. Table 2 demonstrates the MBD of serum at 1 and 6 h, which correspond to the serum concentrations in Table 1.

As can be seen from Tables 1 and 2, 18,750 U of procaine penicillin G (a dose that cured no rabbits) gave mean serum concentrations of penicillin at 1 h that were 0.7 μg/ml (range in 11 rabbits, <0.2 to 2.0) and a median MBD of 1/4 to 1/8. In rabbits treated with 37,500 U (the smallest dose that resulted in cure in any animals), the mean serum concentration was 1.6 μg/ml (range in 12 rabbits, 0.8 to 2.4) at 1 h with a median MBD of 1/8 to 1/16. With 75,000 U (the minimal dose that cured all animals), the mean serum concentration at 1 h was 2.1 μg/ml (range in 31 rabbits, 0.9 to 3.4) and the MBD was 1/16. At 6 h the serum concentrations of penicillin were not measurable (<0.2 μg/ml) for doses of 18,750, 37,500, and 75,000 U; the median MBDs were only 1/2 or less for these doses.

DISCUSSION

Procaine penicillin G has been shown effective in the treatment of penicillin-susceptible streptococcal endocarditis in rabbits (3, 5, 6, 8, 11). With the addition of streptomycin or gentamicin, a synergistic effect has been noted (5, 8, 11). The doses of procaine penicillin used in these studies have been 25 mg/kg (equivalent to about 80,000 U/rabbit) every 12 h (5), 300,000 U/rabbit every 12 h (3, 6, 11), and 250 mg/kg (equivalent to about 800,000 U/rabbit once a day [8]). The penicillin dosage required to treat streptococcal endocarditis will obviously depend in part on the susceptibility to penicillin of the streptococcal strain responsible for the infection. In the present study and one other (3), the minimal bactericidal concentration of penicillin G for the streptococcal strain under study was 0.1 μg/ml as compared with 0.02 μg/ml in two reports (5, 8) and 0.06 μg/ml in two others (6, 11).

TABLE 1. *Concentration of penicillin in serum after intramuscular administration of procaine penicillin*[a]

Dose (U)	Serum levels (mean μg/ml ± SD) after:			
	1 h	2 h	4 h	6 h
18,750	0.7 ± 0.8 (0.4)[b]	<0.2	<0.2	<0.2
37,500	1.6 ± 0.7 (1.5)	<0.2	<0.2	<0.2
75,000	2.1 ± 1.0 (2.0)	1.0 ± 0.1 (0.9)	<0.2	<0.2

[a] A penicillin concentration of 0.2 μg/ml was the lowest that could be measured.

[b] Numbers in parentheses are median values.

TABLE 2. *MBD of serum after intramuscular injection of procaine penicillin*

Dose in units	MBDs after:	
	1 h	6 h
18,750	<1/2, 1/2, 1/2, 1/4, 1/8, 1/8, 1/16, 1/32 (median, 1/4 to 1/8)	<1/2, <1/2, <1/2, <1/2, <1/2, <1/2, <1/2, 1/2 (median, <1/2)
37,500	1/4, 1/8, 1/8, 1/8, 1/16, 1/32, 1/32, 1/32 (median, 1/8 to 1/16)	<1/2, <1/2, <1/2, <1/2, 1/2, 1/2, 1/2, 1/2 (median, <1/2 to 1/2)
75,000	1/8, 1/16, 1/16, 1/16, 1/16, 1/32, 1/32, 1/32 (median, 1/16)	<1/2, <1/2, <1/2, 1/2, 1/2, 1/8, 1/8, 1/8 (median, 1/2)

In the present studies, regimens consisting of 18,750 U of procaine penicillin or less given every 12 or every 6 h were not sufficient to treat streptococcal endocarditis, as demonstrated by the 50% incidence of positive blood cultures and large numbers of organisms in vegetations after 10 days of treatment.

Doses of 37,500 U (or greater) of penicillin every 6 h were effective in rapidly lowering titers of streptococci in vegetations, and streptococci could not be isolated from most rabbits treated with 37,500 U or more for 5 days. Ten days of treatment resulted in no streptococci isolated from vegetations of rabbits treated with 75,000 U and, in fact, resulted in cure of infection, as demonstrated by lack of relapse after discontinuing penicillin. With 37,500 U, 86% relapsed after 5 days of treatment, 56% after 10 days, and only 13% after 20 days of therapy. Thus, 37,500 U every 6 h was the lowest dose tested that cured most animals, and 75,000 was the lowest dose tested that cured all animals after a 20-day, or shorter, period of therapy.

These studies demonstrated not only the necessity of an adequate dose of penicillin, but also the importance of prolonged therapy. Twenty days of 37,500 U every 6 h was more effective in preventing relapse than 5 or 10 days of treatment; 6 of 7 relapsed with 5 days of treatment; 5 of 9 relapsed with 10 days; and 1 of 8 relapsed after 20 days of therapy ($P < 0.05$ by the chi-square test only for 5 days versus 20 days). It is of interest that 150,000 U of procaine penicillin daily was more effective when given every 6 h rather than every 12 h. The mean $\log_{10}$ CFU ± SD was 1.7 ± 1.0 per g for the former group as compared with 4.5 ± 3.0 per g for the latter group ($P < 0.01$).

The mean serum concentration 1 h after injection of 18,750 U of penicillin was 0.7 μg/ml, which correlates well with the MBD of 1/4 to 1/8. The mean serum concentration for animals treated with 37,500 U of penicillin was 1.6 μg/ml, which agrees with the median MBD of 1/8 to 1/16. The mean serum concentration for animals treated with 75,000 U was 2.1 μg/ml, which correlates well with the median MBD of 1/16. These data suggest that a median MBD of at least 1/8 was necessary to cure endocarditis; this finding agrees with what has been generally stated in the literature (1, 2, 7, 9). It is clear that trough levels were not critical, since half of the rabbits treated with 37,500 U of penicillin every 6 h did not have measurable MBDs at 6 h, and the rest measured only 1/2.

ACKNOWLEDGMENT

We thank William Kobasa for expert technical assistance.

LITERATURE CITED

1. **Barry, A. L., and L. D. Sabath.** 1974. Special tests: bactericidal activity and activity of antimicrobics in combination, p. 431–435. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
2. **Bryan, C. S., S. R. Marney, Jr., R. H. Alford, and R. E. Bryant.** 1975. Gram-negative bacillary endocarditis. Interpretation of the serum bactericidal test. Am. J. Med. 58:209–215.
3. **Carrizosa, J., W. D. Kobasa, and D. Kaye.** 1975. Antagonism between chloramphenicol and penicillin in streptococcal endocarditis in rabbits. J. Lab. Clin. Med. 85:307–311.
4. **Durack, D. T., P. B. Beeson, and R. G. Petersdorf.** 1973. Experimental bacterial endocarditis. III. Production and progress of the disease in rabbits. Br. J. Exp. Pathol. 54:142–151.
5. **Durack, D. T., L. L. Pelletier, and R. G. Petersdorf.** 1974. Chemotherapy of experimental streptococcal endocarditis. II. Synergism between penicillin and streptomycin against penicillin-sensitive streptococci. J. Clin. Invest. 53:829–833.
6. **Hook, E. W., III, and M. A. Sande.** 1974. Role of the vegetation in experimental *Streptococcus viridans* endocarditis. Infect. Immun. 10:1433–1438.
7. **Mandell, G. L.** 1976. The laboratory in diagnosis and management, p. 155–166. *In* D. Kaye (ed.), Infective endocarditis. University Park Press, Baltimore.

8. **Pelletier, L. L., and R. G. Petersdorf.** 1976. Chemotherapy of experimental streptococcal endocarditis. V. Effect of duration of infection and retained intracardiac catheter on response to treatment. J. Lab. Clin. Med. 87:692-702.
9. **Quinn, E. L., and F. Cox.** 1970. Bacterial endocarditis: based upon experience with 203 patients, p. 260-274. *In* B. Kagan (ed.), Antimicrobial therapy. W. B. Saunders Co., Philadelphia.
10. **Ries, K., M. E. Levison, and D. Kaye.** 1973. Clinical and in vitro evaluation of cefazolin, a new cephalosporin antibiotic. Antimicrob. Agents Chemother. 3:168-174.
11. **Sande, M. A., and R. G. Irvin.** 1974. Penicillin-aminoglycoside synergy in experimental *Streptococcus viridans* endocarditis. J. Infect. Dis. 129:573-576.