

Ampicillin Therapy of Experimental Enterococcal Endocarditis

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In rabbits with experimental enterococcal endocarditis, subcutaneously implanted perforated polyethylene chambers were used for ampicillin administration by intra-chamber injection. A total of 21 days of intra-chamber ampicillin therapy sterilized vegetations of 14 out of 14 rabbits with experimental enterococcal endocarditis. In rabbits treated for less than 21 days, the duration of therapy and quantitative vegetation cultures were inversely related. Peak serum minimal bactericidal titers were $\geq 1:8$ in 94% of the determinations. Trough serum minimal bactericidal titers were $\leq 1:2$. The mean trough serum ampicillin concentration (2.6 $\mu\text{g}/\text{ml}$) was greater than the minimal bactericidal concentration of ampicillin for the infecting enterococcus and less than the mean trough chamber fluid ampicillin concentration (3.7 $\mu\text{g}/\text{ml}$). Relatively prolonged therapy with intra-chamber injections seemed to be well tolerated. Combination drug therapy of enterococcal endocarditis may not always be required. The maintenance of serum minimal bactericidal titers $\geq 1:8$ throughout the therapy of endocarditis, as is often recommended, may be unnecessary.

Subcutaneously implanted perforated chambers have been used to study the dynamics of systemically administered antibiotics in serum and in the fluid that accumulates within the chambers. Some investigators believe that chamber fluid (CF) is similar to interstitial fluid and that CF antibiotic levels approximate tissue levels of antibiotics (3, 10, 11). The injection of ampicillin directly into a subcutaneous chamber (intra-chamber [IC] injection) resulted in more prolonged serum and CF ampicillin concentrations than intramuscular injection (11). The aims of the present study were to determine if experimental enterococcal endocarditis (EEE) could be cured with ampicillin and to use subcutaneous chambers containing CF for IC ampicillin administration and for the monitoring of CF antibacterial activity.

MATERIALS AND METHODS

Experimental endocarditis. Rabbits with subcutaneously implanted polyethylene chambers, as described previously, were studied (11). Experimental endocarditis was produced by a modification of the method of Weinstein and Lentnek (12). Briefly, rabbits with implanted subcutaneous chambers were anesthetized with intravenous sodium pentobarbital and ether by inhalation. The right carotid artery was then exposed and ligated cephalad. A sterile polyethylene catheter was inserted into the artery and advanced proximally into the left ventricle. The catheter was clamped and sutured to the artery and left in place for

3 days. Rabbits were then given an intravenous injection of 10^8 colony-forming units (CFU) of *Streptococcus faecalis*, and the catheter was removed immediately. Rabbits subsequently treated with ampicillin were considered to have EEE if the quantitative blood culture obtained 24 h after the intravenous injection of *S. faecalis* grew ≥ 100 CFU of *S. faecalis* per ml of blood. Preliminary studies and studies of others indicated that bacteremias of this magnitude at 24 h accurately predict colonization of sterile platelet-fibrin vegetations by the infecting enterococcus (5). Blood for culture and for serum ampicillin assays and bactericidal activity determinations was obtained from marginal ear veins of rabbits.

Infecting organism. The enterococcus used in all experiments was obtained from a blood culture of a patient with endocarditis. The minimal inhibitory concentration of ampicillin was 1.0 $\mu\text{g}/\text{ml}$. The minimal bactericidal concentration of ampicillin was 2.0 $\mu\text{g}/\text{ml}$. All bacteria recovered from blood cultures and cardiac vegetations were identified by the formation of typical colonies on blood agar, the ability to blacken bile-esculin agar, the tolerance to 6.5% sodium chloride broth, and the inability to produce catalase.

Administration of ampicillin. Preliminary studies in which normal rabbits with implanted chambers received graded single doses of IC ampicillin suggested that 150 mg/kg given by an IC injection every 12 h would produce continuous serum and CF ampicillin levels in excess of the minimal bactericidal concentration of ampicillin for the infecting enterococcus. Treatment of rabbits with IC ampicillin doses of 150 mg/kg every 12 h was begun 24 h after an intravenous injection of enterococci. Therapy was continued until death or for 21 days, a duration of therapy associated with a high cure rate in previous studies of EEE (12). Only one chamber was used for IC ampicillin injection in

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each rabbit. EEE was produced in six groups of four to six rabbits with therapy begun on different days. Each of the six groups included one or two untreated controls.

Assay of ampicillin and determination of bactericidal activity. Serum and CF ampicillin concentrations were determined by a disk agar diffusion technique (11). For CF ampicillin assays, standard curves were determined with pooled CF. For serum assays, standard curves were determined with pooled rabbit serum. Minimal bactericidal titers (MBTs) of serum and CF were determined by a modification of the method of Barry and Sabath (1). A 0.5-ml aliquot of each serum and CF specimen was diluted in twofold steps in tubes containing 0.5 ml of normal rabbit serum or CF, and to this, 0.5 ml of Mueller-Hinton broth containing 10^6 CFU of the infecting enterococcus 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 MBT was the highest dilution of serum or CF resulting in fewer than 10 colonies on the plate after 48 h of incubation at 37°C . This represents at least a 99.9% kill rate (1). Student's *t* test was used for statistical comparisons.

Monitoring of therapy. Rectal temperatures were determined twice daily with an electronic thermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Once daily, just before the administration of ampicillin, 0.5 ml of blood was withdrawn aseptically from an ear vein and mixed with 10 ml of heated tryptic soy agar (Difco Laboratories, Detroit, Mich.) for pour plate cultures. Colonies were counted after 24 h of incubation at 35°C . No attempt was made to inactivate ampicillin. A blood culture was obtained from any rabbit with a temperature $\geq 40.0^\circ\text{C}$. Daily cultures were discontinued if a treated rabbit had a temperature $\leq 40.0^\circ\text{C}$ and two consecutive negative cultures. Trough and peak serum ampicillin concentrations, based on preliminary studies, were determined between 4 and 7 days and again between 11 and 14 days after the initiation of therapy by obtaining blood specimens just before and 1 h after a dose of IC ampicillin. Serum MBTs were determined with the same blood specimens. Similarly, CF was obtained just before and 6 h after a dose of IC ampicillin for determinations of trough and peak CF ampicillin concentrations and MBTs. CF for these determinations was always obtained from the chamber on the side of the rabbit opposite from the one used for the IC ampicillin injection. Serum and CF ampicillin levels and MBTs were again determined during the week 3 of therapy in rabbits in which a previous peak serum MBT was $<1:8$.

Posttherapy determinations. Some rabbits with EEE were treated for 21 days and then observed for 3 weeks posttherapy with daily temperature measurements. Blood cultures were obtained twice weekly and whenever a rabbit had a temperature $\geq 40.0^\circ\text{C}$. Rabbits died during therapy or were sacrificed 1 or 22 days after the completion of therapy by an intravenous administration of sodium pentobarbital. The heart was removed, the left ventricle was opened, and aortic valve vegetations were aseptically removed and weighed. Vegetations were suspended in 9.9 ml of sterile saline containing 1,000 U of penicillinase and

then homogenized in a tissue grinder. Serial 10-fold dilutions were incorporated into pour plates of tryptic soy agar and incubated for 24 h at 37°C , and the results were recorded as the CFU per gram of vegetation. The original vegetation suspension was also cultured qualitatively by adding 10 ml of Mueller-Hinton broth to the suspension, incubating at 37°C for 48 h, and then subculturing onto blood agar at 24 and 48 h.

RESULTS

Untreated rabbits. Eight out of eight untreated rabbits with EEE died 3, 3, 6, 7, 7, 8, 9, and 11 days, respectively, after infection. Peak temperatures after infection varied from 40.6 to 41.3°C . Temperatures decreased to less than 39.5°C in three rabbits on the day before death or on the day of death. After day 2 of infection, the numbers of CFU of enterococci per ml of blood increased with each day of survival. Final quantitative blood cultures ranged from 820 to over 5,000 CFU/ml. Aortic valve vegetations weighed 35 to 148 mg and contained $\geq 8 \times 10^8$ CFU/g of vegetation. Results of vegetation cultures in untreated and treated rabbits are shown in Fig. 1.

IC ampicillin therapy. A total of 14 rabbits with EEE were treated for 21 days. Bacteremias cleared within 1 to 6 days after the initiation of therapy. Nine rabbits were sacrificed 24 h after the last ampicillin dose, and five were sacrificed 3 weeks later. All 14 had sterile vegetations. The five rabbits observed for relapse all remained

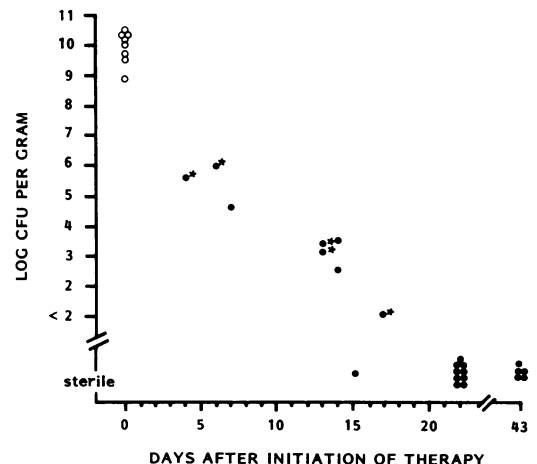


FIG. 1. Log_{10} CFU of enterococci in aortic valve vegetations of untreated rabbits with endocarditis (○) and of rabbits treated for 4 to 21 days with IC ampicillin doses of 150 mg/kg every 12 h (●). Each circle represents a quantitative vegetation culture from one rabbit. Closed circles identified by an asterisk indicate rabbits that died in association with diarrhea during therapy.

afebrile and had negative blood cultures. Nine other rabbits died from 4 to 17 days after the initiation of therapy. In five of these, death was associated with acute severe diarrhea. In rabbits treated for less than 21 days, there was an inverse relationship between the number of days of therapy and the number of CFU on vegetation cultures (Fig. 1). Vegetations of one rabbit treated for 15 days were sterile, whereas one rabbit treated for 17 days still had low concentrations of enterococci in vegetations, with no growth on quantitative cultures.

Serum and CF ampicillin concentrations and bactericidal titers. Serum and CF ampicillin concentrations in seven normal rabbits after single doses of IC ampicillin of 150/per kg are shown in Table 1. Peak and trough serum and CF ampicillin concentrations and MBTs in rabbits treated for EEE are shown in Table 2. Peak serum MBTs $\geq 1:8$ were achieved in 28 out of 30 determinations. Trough serum ampicillin concentrations were greater than the minimal bactericidal concentration of ampicillin for the infecting enterococcus in 27 out of 30 determinations. CF ampicillin concentrations and MBTs were less than peak serum values. The mean trough CF ampicillin concentration, however, exceeded the mean trough ampicillin concentra-

tion in serum, as previously shown (11). Peak serum ampicillin concentrations in rabbits with EEE (mean, 16.9 $\mu\text{g/ml}$) were significantly lower than peak serum ampicillin concentrations in normal rabbits (mean, 23.4 $\mu\text{g/ml}$; $P < 0.05$). In contrast, peak CF ampicillin concentrations in rabbits with EEE (mean, 8.8 $\mu\text{g/ml}$) were higher than peak CF ampicillin concentrations in normal rabbits (mean, 5.2 $\mu\text{g/ml}$; $P < 0.025$).

DISCUSSION

Peak serum ampicillin concentrations after single doses of IC ampicillin of 150 mg/kg in normal rabbits were not significantly different from peak levels obtained after single IC ampicillin doses of 50 mg/kg in a previous study (11). The duration of serum ampicillin levels in excess of the minimal bactericidal concentration of the infecting enterococcus was greater after the higher dose, however, and influenced the dosage chosen for therapy in this study. The finding of lower peak serum ampicillin levels in rabbits with EEE as compared with normal rabbits is difficult to interpret because normal rabbits treated with multiple doses of IC ampicillin were not studied.

Relatively prolonged IC ampicillin therapy seemed to be well tolerated. Despite the administration of up to 42 twice-daily ampicillin injections into the same chamber there was no evidence of inflammation of adjacent skin or subcutaneous tissue. Injections appeared to cause little or no pain. Rabbits did not usually struggle or withdraw during IC ampicillin injections. During therapy, the CF within the chamber used for IC injections often became cloudy due to a moderate influx of leukocytes. The infection of chambers used for therapy or monitoring was not a problem.

In this study, 5 out of 23 rabbits (22%) receiving IC ampicillin therapy died in association with severe diarrhea. Enterocolitis due to toxin-producing *Clostridium difficile* may have occurred, as has been suggested by other reports (6). However, no stool cultures or toxin assays were performed.

The present study demonstrated the possibility of curing EEE with ampicillin alone, supporting other data suggesting that the therapy of enterococcal endocarditis with a combination of penicillin or ampicillin and an aminoglycoside may not always be necessary (2, 7-9). Also, results demonstrated the feasibility of delivering the relatively prolonged course of therapy required for cure via the IC route of administration. Finally, this treatment regimen sterilized vegetations despite trough serum MBTs $\leq 1:2$, suggesting that the maintenance of serum MBTs

TABLE 1. Serum and CF concentrations of ampicillin after IC administration of 150 mg/kg in seven normal rabbits

Time (h) after IC administration	Ampicillin concn ^a ($\mu\text{g/ml}$) in:	
	Serum	CF
0.5		0.2 \pm 0.1
1	23.4 \pm 4.8	0.8 \pm 0.3
2		2.0 \pm 0.6
4	13.9 \pm 2.4	3.9 \pm 1.2
6		5.2 \pm 1.6
8	7.9 \pm 1.6	4.9 \pm 1.3
12	3.4 \pm 0.9	3.5 \pm 1.3
16	2.1 \pm 0.7	2.7 \pm 0.6
24	1.6 \pm 0.4	2.5 \pm 0.3

^a Mean \pm the standard error of the mean.

TABLE 2. Serum and CF ampicillin concentrations and reciprocal MBTs (1/MBT) during ampicillin therapy of EEE

Time	Specimen	Ampicillin concn ^a ($\mu\text{g/ml}$)	1/MBT ^b
Peak	Serum	16.9 \pm 2.3	16 (4- \geq 64)
	CF	8.8 \pm 1.4	4 (<2-16)
Trough	Serum	2.6 \pm 0.6	<2 (<2-2)
	CF	3.7 \pm 1.1	<2 (<2-4)

^a Mean \pm the standard error of the mean.

^b Median (range).

$\geq 1:8$ throughout the therapy of endocarditis, as is often recommended (4), may be unnecessary (9).

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid from the American Heart Association, Indiana Affiliate, Inc.

James Teipen provided excellent technical assistance and Dorothy Jones typed the manuscript.

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