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Treatment of Streptomycin-Susceptible Enterococcal Experimental Endocarditis with Combinations of Penicillin and Low- or High-Dose Streptomycin

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We used two strains of streptomycin-susceptible enterococci (MIC, 64 and 128 μg of streptomycin per ml, respectively) isolated from patients with infective endocarditis. When combined with penicillin, 20 μg of streptomycin per ml killed both strains synergistically in vitro whereas combinations of 5 and 10 μg of streptomycin per ml did not act synergistically against either strain. By using the rabbit model of enterococcal experimental endocarditis, animals were treated for 3 days with procaine penicillin (1.2×10^6 U intramuscularly three times daily) together with low-dose streptomycin (3.5 mg/kg) or high-dose streptomycin (10 mg/kg) intramuscularly three times daily. The peak concentrations of streptomycin in serum at 0.5 h were 9.2 and 26.8 μg/ml in the low- or high-dose group, respectively. When combined with procaine penicillin, both dosages of streptomycin were more effective ($P < 0.01$) than procaine penicillin alone for the treatment of enterococcal experimental endocarditis. There was no significant difference in the efficacy of procaine penicillin plus low-dose streptomycin versus procaine penicillin plus high-dose streptomycin therapy of enterococcal experimental endocarditis.

Antimicrobial therapy for enterococcal infective endocarditis consists of penicillin combined with an aminoglycoside administered parenterally for 4 to 6 weeks. In patients with streptomycin-susceptible enterococcal endocarditis (MIC, <2,000 μg of streptomycin per ml), the use of streptomycin is preferred (2, 5, 6, 8, 9, 12). The usual dosages of streptomycin result in peak and trough concentrations in serum of 25 to 35 and 5 to 15 μg/ml, respectively, that are associated with vestibular toxicity in approximately 20% of patients treated for 4 weeks (13). In a previous study of streptomycin-susceptible enterococci, we observed synergistic killing in vitro by combinations of penicillin together with either 20 or 10 μg of streptomycin per ml, but the magnitude of killing achieved by 20 μg of streptomycin per ml was significantly greater ($P < 0.01$) than that by 10 μg of streptomycin per ml (7). If a lower dosage of streptomycin were equally effective as a higher dosage in vivo, the risk of streptomycin-associated vestibular toxicity might be less. The purpose of this study was to determine the efficacy of penicillin combined with a low dose of streptomycin compared with a higher dose of streptomycin in the treatment of streptomycin-susceptible enterococcal experimental infective endocarditis in rabbits.

MATERIALS AND METHODS

Organisms. Two strains of *Streptococcus faecalis* isolated from patients with infective endocarditis susceptible to 64 and 128 μg of streptomycin per ml were used. The penicillin MIC was 1 μg/ml and the MBC was >128 μg/ml for both strains of enterococci.

In vitro synergy tests. Inocula of approximately 10^7 CFU/ml were prepared from an overnight culture of the two strains of enterococci grown in Mueller-Hinton broth (MHB). The overnight cultures were diluted to 1:20 in MHB containing antibiotics singly or in combination. Concentrations used were 20 μg of penicillin per ml alone or in combination with 5, 10, or 20 μg of streptomycin per ml. After 0, 4, and 24 h of incubation at 37°C, 0.2-ml samples were removed and dilated 10-fold in MHB. Samples (0.1 ml) from each dilution were spread over the surface of tryptic soy agar plates (pH 7.35; Difco Laboratories, Detroit, Mich.) containing 10^6 U of penicillinase and incubated at 37°C. After 48 h, colonies were counted, and the CFUs/ml were calculated. Experiments were performed in triplicate, and results are expressed as mean $\log_{10}$ CFU/ml. Synergy was defined as at least a 100-fold increase in killing after 24 h of incubation by a combination of antimicrobial agents compared with that achieved by either antimicrobial agent alone (11).

Rabbit model of endocarditis. Experimental aortic valve endocarditis was established in New Zealand white rabbits (weight, approximately 2 kg) by a modification of the model described by Garrison and Freedman (4). Animals were anesthetized with a 1:10 mixture of ketamine, and the right carotid artery was exposed through a midline excision in the neck. The artery was ligated distally, and a sterile polyethylene catheter (Intramedic; PE90; Clay Adams) was advanced proximally through a nick in the artery. The distal tip of the catheter was connected to a pressure-sensitive monitoring device to ensure proper placement of the proximal catheter tip into the left ventricle. When the catheter tip crossed the aortic valve and entered the left ventricle, advancement was stopped and the distal end of the catheter was sealed and tied to the carotid artery, and the incision was closed over the catheter with surgical clips. The catheter was left in place throughout the duration of the experiment. Enterococci were inoculated into tryptic soy broth and incubated overnight. A fresh culture of enterococci was prepared daily for animal inoculation. A 1-ml amount of broth containing 10^6 to 10^7 CFU of enterococci was injected

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TABLE 1. Concentration of streptomycin in serum in uninfected rabbits

Time interval after administration of dosage (h)	Concn (μg/ml) of streptomycin in serum at the following administration dosages[a]:	
	3.5 mg/kg body weight	10 mg/kg body weight
0.5	9.1 ± 0.9	25.7 ± 1.3
1	6.9 ± 0.7	18.2 ± 0.9
2	2.6 ± 0.4	8.1 ± 1.1
4	1.2 ± 0.7	2.3 ± 0.4
8	<1	<1
12	<1	<1

[a] Values are means ± standard deviations.

through a peripheral ear vein 24 h after insertion of the catheter.

Blood cultures. A 1-ml amount of blood was obtained 24 h after infection and just before initiation of antimicrobial therapy. The blood specimen was mixed with 20 ml of molten enterococcal agar containing bile esculin and sodium chloride. Plates were incubated for 18 to 24 h at 37°C, and the presence of enterococci was interpreted as indicative of infective endocarditis. Experimental endocarditis resulted in infection of 98.6% of the animals.

Treatment groups. Antimicrobial therapy was initiated 24 h after injection of enterococci. Antimicrobial agents were administered intramuscularly (i.m.) three times daily (TID; 8 a.m., noon, 4 p.m.) for 3 days. Animals were placed in the following treatment groups. (i) Control consisted of 20 animals (10 mice infected with each strain) that received no antimicrobial therapy. (ii) A total of 36 mice (18 infected with each strain) received 1.2×10^6 U of procaine penicillin (Wyeth Laboratories) i.m. TID. (iii) A total of 36 (18 mice infected with each strain) received procaine penicillin (1.2×10^6 U i.m. TID) plus streptomycin (3.5 mg/kg i.m. TID). (iv) A total of 36 animals (18 mice infected with each strain) received procaine penicillin (1.2×10^6 U i.m. TID) plus streptomyin (10 mg/kg i.m. TID).

After the day 3 of treatment, animals were sacrificed with a lethal intravenous injection of sodium pentothal at least 12 h after the last dosage of antibiotic(s) was administered. The chest was opened, the heart was excised and opened, and the aortic valve vegetations were removed aseptically. The vegetations were weighed and homogenized in 0.1 ml of sterile saline solution in a sterile mortar and pestle. The homogenate was suspended in 9.9 ml of MHB containing 10^6 U of penicillinase. Duplicate 1-ml samples of the homogenate were mixed with 20 ml of molten enterococcal agar containing bile esculin and sodium chloride and incubated at 37°C for 72 h. Colonies were counted and results are expressed as mean $\log_{10}$ CFU of enterococci per g of valve vegetation.

Serum concentrations of streptomycin in serum. Twelve uninfected animals received streptomycin dosages of 3.5 and 10 mg/kg i.m., and blood samples were obtained from each animal at 0.5, 1, 2, 4, 8, and 12 h after administration. The highest streptomycin concentrations in serum occurred at 0.5 h (Table 1). There were no overlaps in the concentrations of streptomycin in serum at 0.5 h in animals treated with low- or high-dose streptomycin.

On day 3 of antimicrobial therapy, blood samples were obtained from infected animals 30 min after administration of procaine penicillin and streptomycin for determination of the streptomycin concentration in serum. The serum specimens were frozen at −20°C. Streptomycin concentrations were determined by high-pressure liquid chromatography (11).

Bactericidal titer in serum. Fractions of serum from the same specimen obtained for antimicrobial assay were frozen at −20°C. Specimens were thawed for determination of the bactericidal titer in serum by previously described methods (11).

Analysis of results. Differences in mean $\log_{10}$ in CFU of enterococci per gram of valve vegetation were analyzed statistically by the Kruskal-Wallis test and the individual rank serum test for differences between pairs (3).

RESULTS

The killing in vitro of the two strains of enterococci by penicillin and streptomycin singly or in combination is shown in Table 2. The standard deviations are not shown because in all instances they were less than 10% of the mean. With both strains, there was at least a 2-log increase in killing at 24 h only with the combination of penicillin and 20 μg of streptomycin per ml compared with that achieved by either drug alone. The combination of penicillin and 5 or 10 μg of streptomycin per ml was more effective in vitro than was either drug tested alone, but these combinations of drugs did not act synergistically in vitro according to our definition of synergy.

The concentrations of streptomycin in serum and the results of treatment of animals with procaine penicillin alone or procaine penicillin plus streptomycin are shown in Table 3. There was a statistically significant difference ($P < 0.01$) between the mean $\log_{10}$ CFU of cardiac valve vegetation per gram between animals treated with procaine penicillin alone and those treated with procaine penicillin combined with either dosage of streptomycin. Compared with the lower dosage of streptomycin, the higher dosage of streptomycin did not result in a statistically significant difference in killing of enterococci in vivo with either strain of enterococci. The median (range) bactericidal titers in serum are shown in Table 4.

DISCUSSION

The results of our vitro study confirm previous observations (7) that penicillin combined with 20 μg of streptomycin per ml was more active in vitro than when combined with 10 μg of streptomycin per ml against strains of streptomycin-

TABLE 2. In vitro activity of penicillin and streptomycin against two strains of enterococci

Antimicrobial agent (concn, μg/ml)	$\log_{10}$ CFU/ml					
	Strain 1			Strain 2		
	0 h	4 h	24 h	0 h	4 h	24 h
Streptomycin (5)	7.2	7.4	8.2	7.2	8.1	8.6
Streptomycin (10)	7.2	7.2	8.4	7.2	8.0	8.5
Streptomycin (20)	7.2	7.4	8.1	7.2	8.4	8.6
Penicillin (20)	7.2	6.8	6.3	7.2	7.3	6.8
Penicillin (20) plus streptomycin at:						
5	7.2	6.4	5.9	7.2	6.1	5.5
10	7.2	5.9	5.3	7.2	6.0	5.6
20	7.2	5.5	3.1	7.2	6.0	3.0
Control	7.2	8.1	8.4	7.2	8.2	8.6

TABLE 3. Results of treatment of experimental enterococcal endocarditis with procaine penicillin combined with low- or high-dose streptomycin

Treatment group	Dosage of[a]:		Strain 1			Strain 2		
	Penicillin (U, 10^6)	Streptomycin (mg/kg)	No. of animals	Mean ± SD streptomycin concn in serum at 0.5 h	Mean ± SD $\log_{10}$ CFU in cardiac valve vegetation/g	No. of animals	Mean ± SD streptomycin concn in serum at 0.5 h	Mean ± SD $\log_{10}$ CFU in cardiac valve vegetation/g
i			10		10.2 ± 0.6	10		10.2 ± 0.3
ii	1.2		18		6.8 ± 0.8[b]	18		6.5 ± 0.5[b]
iii	1.2	3.5	18	9.2 ± 1.3	5.1 ± 1.1[c]	18	9.1 ± 0.7	4.7 ± 0.9[c]
iv	1.2	10.0	18	26.8 ± 1.1	5.3 ± 0.6[d]	18	25.9 ± 1.8	5.1 ± 1.0[d]

[a] Drug was administered i.m. TID.
[b] $P < 0.001$ (procaine penicillin versus control).
[c] $P < 0.01$ (procaine penicillin-streptomycin versus procaine penicillin).
[d] P not significant (procaine penicillin-streptomycin [3.5 mg/kg] versus procaine penicillin-streptomycin [10 mg/kg]).

susceptible enterococci. However, the results of our study of experimental enterococcal endocarditis do not demonstrate a superiority in efficacy in vivo of higher-dose streptomycin over low-dose streptomycin after 3 days of treatment. A discrepancy between in vitro and in vivo killing of enterococci has been reported elsewhere (2).

The results of in vitro synergy tests of enterococci are influenced by the inoculum size of the enterococci tested (1). We chose to use a larger inoculum size of enterococci than that which is frequently used (9, 10) because the larger inoculum more closely simulates the number of microorganisms present in experimental cardiac valve vegetations.

The mean peak concentration of streptomycin in serum (26.3 μg/ml) observed in animals treated with high-dose streptomycin was similar to the mean peak streptomycin concentration in serum in patients with streptomycin-susceptible enterococcal endocarditis treated at Mayo Clinic (13). We are unaware of published data which compare the efficacy of streptomycin to that of gentamicin for the treatment of patients with endocarditis caused by streptomycin-susceptible enterococci. The relapse rate among patients with streptomycin-resistant enterococcal endocarditis was higher following treatment with gentamicin than that observed among patients treated with streptomycin for streptomycin-susceptible endocarditis (13). In this same study, vestibular toxicity occurred more commonly in patients treated with streptomycin, and nephrotoxicity was observed only in patients who received gentamicin therapy. We believe that streptomycin and gentamicin are probably equivalent in efficacy for the treatment of patients with streptomycin-susceptible enterococcal endocarditis. However, until more data are available concerning the use of gentamicin in these patients, we agree with Sande and Scheld (8) that streptomycin currently is the aminoglycoside of choice for the treatment of patients with streptomycin-susceptible enterococcal endocarditis. We were unable to find published data concerning the treatment of patients with dosages of streptomycin which resulted in peak concentrations in serum similar to those observed in animals treated with low-dose streptomycin in our study. The lack of a significant difference in the in vivo killing of enterococci in experimental endocarditis in our study by low-dose compared with high-dose streptomycin and the potential for less frequent streptomycin-associated vestibular toxicity in humans treated with lower dosages of streptomycin suggest that clinical trials of the treatment of patients with lower streptomycin dosages may be warranted.

TABLE 4. Results of bactericidal titer tests in serum

Antimicrobial therapy i.m. 3 times daily	Median bactericidal titer in serum (range)	
	Strain 1	Strain 2
Procaine penicillin (1.2 × 10^6 U)	1:2 (undiluted-1:2)	1:2 (undiluted-1:2)
Procaine penicillin (1.2 × 10^6 U) plus streptomycin (3.5 mg/kg)	1:8 (1:2-1:256)	1:8 (1:4-1:256)
Procaine penicillin (1.2 × 10^6 U) plus streptomycin (10 mg/kg)	1:8 (1:4-1:256)	1:8 (1:4-1:64)

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