

Experimental Endocarditis Caused by *Streptococcus sanguis*: Single and Combined Antibiotic Therapy

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The effectiveness of penicillin G, fosfomycin, and cefoxitin alone and in combination was studied in vitro and in the treatment of left-sided *Streptococcus sanguis* endocarditis in rabbits. In vitro, the combinations penicillin G plus fosfomycin, penicillin G plus cefoxitin, and fosfomycin plus cefoxitin were synergistic or partially synergistic for *S. sanguis*. Therapy with the combinations was more effective in eradicating the species from cardiac vegetations than was that with each antibiotic used alone.

Streptococcus viridans is the pathogen most frequently isolated from hemocultures of patients with bacterial endocarditis. The combination of penicillin and aminoglycoside has a synergistic effect against *S. viridans* in vitro and in vivo, as shown by Durack et al. (3), Pelletier and Petersdorf (9), and Sande and Irvin (10). Patients with penicillin allergy should receive vancomycin or a cephalosporin with or without the addition of streptomycin (2, 6, 11). A treatment with less potential toxicity would be a useful alternative for patients allergic to penicillin or suffering renal impairment.

This paper reports on the effectiveness of penicillin G (PG), cefoxitin (Cx), fosfomycin (Fm), and combinations of these drugs in the treatment of experimental endocarditis induced by *Streptococcus sanguis* in rabbits.

MATERIALS AND METHODS

Experimental model. White New Zealand rabbits of either sex, weighing 2 kg \pm 200 g, were used. They were anesthetized with Ketolar (Parke-Davis), 10 mg/kg, using Combelen (Bayer), 0.5 ml per rabbit, as a preanesthetic. Endocarditis was produced in rabbits by the method of Garrison and Freedman (5). Animals were catheterized on different days, but untreated rabbits were included each day as a control.

The right carotid artery was cannulated until the left ventricle was reached, using a polyethylene (Elecath) catheter with exterior and interior diameters of 1 and 0.6 mm, respectively. Catheter and carotid were ligated, and the skin incision was sutured.

Forty-eight hours after cannulation, the rabbits were inoculated in the marginal ear vein with approximately 10^8 colony-forming units (CFU) of streptococci in a 1-ml volume of an overnight culture in brain heart infusion broth adjusted to an optical density of 0.22 in a Spectronic 20 spectrometer.

Hemocultures were obtained 3 days after infection and just before postmortem examination. Blood was taken from the marginal ear vein; 0.1 ml was plated in

Columbia blood agar, and 1 ml was incubated in brain heart infusion broth.

Test strain. A strain of *S. viridans* subsp. *sanguis* serotype II (49678 RyC), isolated from a patient with endocarditis in the Ramón y Cajal Hospital, was used to produce the infection. The minimal inhibitory concentrations for this organism, as determined by serial dilution techniques using Mueller-Hinton broth, were as follows: PG, 0.3 μ g/ml; Cx, 4 μ g/ml; and Fm, 64 μ g/ml.

Interaction tests of the combinations PG+Fm, PG+Cx, and Fm+Cx against this and 16 other strains of *S. viridans* were done by the checkerboard method, using Mueller-Hinton agar. Interactions were classified as synergistic or partially synergistic when fractional inhibitory concentration indices were ≥ 0.5 or ≥ 0.75 , respectively (7).

Bacterial killing. Time-kill curves of PG (0.15 μ g/ml), Cx (2 μ g/ml), Fm (32 μ g/ml), and the combinations for *S. sanguis* were performed in Mueller-Hinton broth. Inocula containing 10^6 organisms per ml were used. Samples were removed after 6, 24, and 48 h of incubation. Serial 10-fold dilutions in sterile saline were made and cultured on Columbia blood agar; the numbers of CFU were counted after 48 h of incubation at 37°C.

Administration of antibiotics. Animals with positive blood cultures were divided into six treated groups and one untreated control group. The latter included 20 rabbits. The antibiotics used and numbers of animals treated (given in parentheses) in the other six groups were as follows: PG (11), Cx (9), Fm (10), PG+Fm (8), PG+Cx (7), and Fm+Cx (7). Whether administered alone or in combination, doses of 100 mg/kg were given intramuscularly twice daily for 5 days, starting 72 h after inoculation.

Vegetations and serum levels. Eighteen cannulated, infected, and treated rabbits were used to study the concentrations of individual drugs in serum and vegetations. Six rabbits were used for each antibiotic. Blood was drawn from the ear vein, and vegetations were removed from the heart. After 3 days of treatment, subgroups of three animals each were sacrificed for this study at each of the two time intervals indicated in Table 4.

Antibiotic concentrations were determined by a diffusion plate method, using *Proteus vulgaris* ATCC 21100 for Fm, *Sarcina lutea* ATCC 9341 for PG, and *Staphylococcus aureus* MB 2786 for Cx.

Vegetations were weighed, homogenized in appropriate buffers and diluted to 1:10. Standard solutions of each antibiotic in rabbit serum and in phosphate buffer (0.1 M), pH 6, for PG and Cx and in tris(hydroxymethyl)aminomethane buffer (0.05 M), pH 8, for Fm were prepared to determine vegetations and serum levels.

Evaluation of infection. Surviving control and treated animals were killed 7 days after infection. Six hours after the last dose of antibiotic, on day 5 of treatment, blood samples were obtained for culture, and the hearts were removed and opened to excise the endocardium vegetations, which were weighed and homogenized in a tissue grinder. Penicillinase was added in cases when penicillin had been used.

The numbers of CFU in blood and vegetations were determined by serial dilution and plating techniques. In addition, 0.5 ml of the undiluted homogenates was cultured in brain heart infusion broth. Vegetations were considered sterile when no growth was seen on plates and there was no turbidity in the tubes incubated at 37°C for 48 h.

Statistical analysis. Student's *t* test was used to determine statistically significant differences in the mean weight of vegetations and in the mean log₁₀ CFU (±standard deviation) of *S. sanguis* per gram of vegetation.

RESULTS

In vitro studies. The susceptibility at critical minimal inhibitory concentration levels of PG, Cx, and Fm of 17 *S. viridans* strains and the synergism of PG+Cx, PG+Fm, and Cx+Fm against these strains are shown in Tables 1 and 2. Eighty-two percent of the strains were susceptible to Cx, 70% were susceptible to PG, and 47% were susceptible to Fm. Synergism, as well as partial synergism, was observed with PG+Fm and Cx+Fm in 94% of the strains and with PG+Cx in 76% of the strains tested by the checkerboard method. The fractionary inhibitory concentration indices against *S. sanguis* were 0.5 for PG+Cx and 0.75 for PG+Fm and Cx+Fm.

The killing curve results for *S. sanguis* (Fig. 1) show that the combinations PG+Fm, PG+Cx, and Cx+Fm produced a remarkable decrease (>4 logs) in the number of viable CFU at 48 h compared with each antibiotic alone. The effect

TABLE 1. *In vitro* susceptibility studies against 17 strains of *S. viridans*

Antibiotic	MIC (critical level, µg/ml) ^a	No. (%) of strains susceptible
PG	≤0.5	12 (71)
Cx	≤16.0	14 (82)
Fm	≤64.0	8 (47)

^a MIC, Minimal inhibitory concentration.

TABLE 2. *In vitro* synergy studies against 17 strains of *S. viridans*

Drug combination	No. (%) of strains showing synergy at FIC ^a index of:	
	≤0.5	≥0.75
PG+Cx	4 (23)	9 (53)
PG+Fm	4 (23)	12 (71)
Cx+Fm	8 (47)	8 (47)

^a FIC, Fractionary inhibitory concentration.

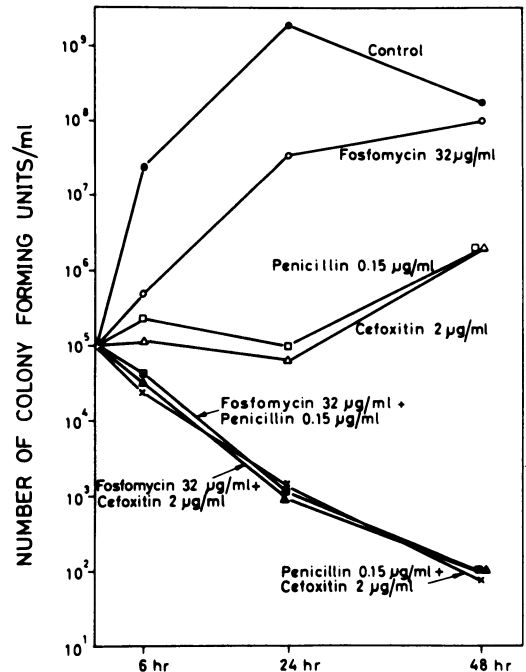


FIG. 1. Killing curves of PG+Fm, PG+Cx, Fm+Cx against *S. sanguis*.

of these combinations was considered to be synergistic according to the criteria of Watanakorn and Bakie (13).

In vivo studies. Results of experimental endocarditis therapy are summarized in Table 3. All rabbits cannulated to evaluate the effectiveness of the treatments tested had positive blood cultures, with mean log₁₀ 2.8 (±0.73) CFU/ml. All of the postmortem blood cultures of the control group were also positive, with mean log₁₀ 3.55 (±0.68) CFU/ml, whereas the postmortem blood cultures of the treated rabbits were negative in 9 of 11 cases for the PG group, in 7 of 9 cases for the Cx group, and in 5 of 10 cases for the Fm group. The postmortem blood cultures of rabbits in the combination treatment groups were sterile.

In the controls there were 90% spontaneous deaths, occurring between days 2 and 7 after

TABLE 3. Results of therapy in experimental streptococcal endocarditis

Experimental group	Response to treatment											
	Therapy (5 days)	No. of rabbits	Bacteremia pretreatment \log_{10} CFU/ml (\pm SD) ^a	Among 7-day fatalities				Among 7-day survivors				Sterile vegetations (no. of rabbits)
				No. of rabbits (mean day to death)	Mean wt of vegetations [mg (\pm SD)]	Vegetations	Blood	No. of rabbits	Mean wt of vegetations [mg (\pm SD)]	Vegetations	Blood	
				No. of rabbits bits	Mean \log_{10} CFU/g (\pm SD)	No. of rabbits bits	Mean \log_{10} CFU/ml (\pm SD)	No. of rabbits bits	Mean \log_{10} CFU/g (\pm SD)	No. of rabbits bits	Mean \log_{10} CFU/ml (\pm SD)	
Control	20	18 (4.5)	2.99 (\pm 0.71)	184.5 (\pm 98.6)	18 (9.07 (\pm 0.35))	18 (3.55 (\pm 0.72))	2 (180 (\pm 40))	2 (9.24 (\pm 0.2))	2 (3.58 (\pm 0.11))	2	2 (3.58 (\pm 0.11))	0
PG	11	3 (4.6)	2.67 (\pm 0.80)	160 (\pm 43.2)	3 (6.74 (\pm 1.08))	1 (1.00)	8 (150 (\pm 77.6))	8 (3.39 (\pm 1.29))	1 (1.87)	1	1 (1.87)	0
Cx	9	2 (5.0)	2.63 (\pm 0.73)	140 (\pm 70.0)	2 (5.94 (\pm 1.09))	1 (1.30)	7 (127.1 (\pm 67.5))	7 (6.84 (\pm 0.81))	1 (2.65)	1	1 (2.65)	0
Fm	10	2 (3.0)	2.87 (\pm 0.61)	85 (\pm 25.0)	2 (7.87 (\pm 0.2))	2 (2.84 (\pm 1.15))	8 (112.5 (\pm 27.2))	8 (5.84 (\pm 1.87))	3 (2.22 (\pm 0.65))	3	3 (2.22 (\pm 0.65))	0
PG+Cx	7	0	2.67 (\pm 0.51)				7 (110.0 (\pm 26.7))	4 (2.38 (\pm 0.39))	0	0	0	3
PG+Fm	8	0	2.58 (\pm 0.88)				8 (91.2 (\pm 24.7))	6 (2.78 (\pm 0.62))	0	0	0	2
Cx+Fm	7	1 (5.0)	3.18 (\pm 0.51)	100	1 (4.73)	0	6 (93.3 (\pm 31.4))	3 (2.49 (\pm 0.57))	0	0	0	3

^a SD, Standard deviation.

infection (average, 4.5 days). In the groups treated with one antibiotic, there were 20 to 27% spontaneous deaths between 3 and 5 days after infection (1 to 3 days of treatment). Finally, in the groups treated with combinations, there was only one death (Cx+Fm group) 5 days after infection (3 days of treatment).

The mean weight of the vegetations of the untreated rabbits (184 mg) was significantly higher than that of the vegetations of the rabbits treated with combinations ($P \geq 0.05$).

Among 7-day survivors, the mean \log_{10} CFU per gram of vegetations in rabbits treated with Fm+Cx was significantly lower than that in animals treated with Fm alone ($P < 0.05$) or Cx alone ($P < 0.001$). The mean \log_{10} CFU per gram of vegetations in the PG+Cx and PG+Fm groups was significantly lower than that in the Fm ($P < 0.005$) and Cx ($P < 0.001$) groups but was not significantly lower than that in the PG group ($P > 0.05$). In this group there were no rabbits with sterile vegetations, whereas 5 of 15 rabbits had sterile vegetations in the PG+Cx and PG+Fm groups.

Levels in serum and vegetations. The levels of the antibiotics in serum and vegetations at various times after day 3 of treatment are shown in Table 4. PG and Cx, but not Fm, penetrated in vegetations at concentrations that exceeded the minimal inhibitory concentration for *S. sanguis* at 1 h after a dose. The diffusions of antibiotics to the vegetations at 1 h in reference to the peak in blood were 20.2, 16.0, and 35.3% for PG, Cx, and Fm, respectively.

DISCUSSION

A previous study showed that Fm interacted synergistically with several antibiotics against different gram-positive and gram-negative bacteria, including a synergistic effect with ampicillin against *Streptococcus* spp. (8). In vitro studies demonstrated synergism for the combinations PG+Fm, PG+Cx, and Fm+Cx by the checkerboard method and bacterial killing curves against the strain of *S. sanguis* used in the current study on experimental endocarditis. The mechanism by which PG and Cx act syn-

ergistically against *S. viridans* is unknown, but there are previous papers (1, 7) that show a synergistic effect of Cx with penicillin, carbenicillin, and mecillinam against *Escherichia coli*, *Enterobacter cloacae*, indole-positive *Proteus* spp., and *Streptococcus faecalis*.

The experimental model tested is similar in many respects to acute endocarditis in humans. The rabbits exhibited fever, bacteriemia, high mortality, and metastatic abscesses in the kidneys. These reactions can be used to test the effectiveness of different antibiotics, administered separately or in combination, and to investigate synergy in vivo.

In this study, when experimental endocarditis caused by *S. sanguis* was treated with Fm and Cx administered alone, the effectiveness of these drugs was lower than that of PG alone, with reductions of 3.4, 2.4, and 5.8, respectively, in mean \log_{10} CFU per gram of vegetation compared with the untreated controls; no sterile vegetations were found. However, when combined pairs of antibiotics were administered, the reductions in the number of viable bacteria per gram of vegetations increased, and sterile vegetations were found in 25% of rabbits for the PG+Fm group and 43% for the PG+Cx and Cx+Fm groups. These results suggest a possible synergistic effect.

In summary, this study shows a good correlation between the in vitro and in vivo results. Partial or total in vitro synergism correlates with the rate of bacterial eradication from vegetations in rabbits treated with combinations of drugs. Moreover, Cx and Fm are bactericidal, have low nephrotoxicity, and diffuse well into extravascular fluids and tissues (4, 12). This study suggests the possibility, therefore, of finding a new alternative for the treatment of human endocarditis caused by *S. viridans*, but further studies are necessary to confirm these promising results.

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TABLE 4. Antibiotic levels in rabbits after intramuscular administration of 100 mg/kg

Antibiotic	Antibiotic level ^a ($\mu\text{g/ml}$) in:						
	Serum				Vegetations		
	15 min	30 min	3 h	6 h	1 h	3 h	6 h
PG		43.5 (± 6.5) ^b		0.9 (± 0.1)	8.8 (± 1.1)		0.7 (± 0.2)
Fm		142.0 (± 17.1)		14.7 (± 1.5)	50.1 (± 6.6)		15.2 (± 1.6)
Cx	134.0 (± 19.3)		3.9 (± 1.0)		21.5 (± 7.0)	<1.0	

^a Average levels of three rabbits.

^b \pm Standard deviation.

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