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Treatment of *Staphylococcus aureus* Endocarditis in Rats with Coumermycin A1 and Ciprofloxacin, Alone or in Combination

CHRISTIAN M. PERRONNE,[1,2] RAFFAEL MALINVERNI,[1] AND MICHEL P. GLAUSER[1]*

Division des Maladies Infectieuses, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland,[1] and Hôpital Claude Bernard, 75019 Paris, France[2]

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The efficacy of a 5-day treatment with coumermycin A1 (hereafter referred to as coumermycin) (at three dosage regimens), with ciprofloxacin, or with coumermycin plus ciprofloxacin was tested in experimental aortic valve endocarditis induced in rats by a strain of methicillin-susceptible *Staphylococcus aureus* and was compared with the efficacy of a 5-day treatment with cloxacillin plus gentamicin. While coumermycin was far less effective than cloxacillin plus gentamicin in reducing the bacterial counts in vegetations ($P < 10^{-8}$), ciprofloxacin was as effective as cloxacillin plus gentamicin. Coumermycin plus ciprofloxacin was less effective than ciprofloxacin alone ($P = 0.01$). For endocarditis induced by two additional methicillin-susceptible *S. aureus* strains, the high-dosage regimen of coumermycin (12 mg/kg every 12 h) had the same low efficacy. Coumermycin-resistant variants of *S. aureus* emerged in most of the vegetations during coumermycin treatment. The ciprofloxacin susceptibility of *S. aureus* was unchanged during ciprofloxacin treatment. The addition of ciprofloxacin to coumermycin in the treatment did not prevent the emergence of coumermycin-resistant variants. Twelve additional *S. aureus* strains isolated from the blood of patients with endocarditis were tested in vitro against coumermycin with precautions to avoid carry-over of the antibiotic. Coumermycin exhibited a bacteriostatic activity at very low concentrations (MIC, <0.004 μg/ml) but only a weak bactericidal activity (MBC for 90% of strains, 8 μg/ml), a finding contrasting with that of others. Furthermore, coumermycin-resistant mutants could be selected in vitro from the 15 *S. aureus* strains tested. These results indicated no evidence in vivo of a synergistic activity of coumermycin and ciprofloxacin. More importantly, these results suggested that coumermycin might not be adequate for the treatment of serious *S. aureus* infections in humans.

Coumermycin A1 (hereafter referred to as coumermycin) is a bis-hydroxycoumarin antibiotic structurally related to novobiocin and isolated from *Streptomyces rishiriensis* (3, 15). Its bacterial target is the B subunit of DNA-gyrase (8, 13). In vitro, a high antibacterial activity is demonstrated by coumermycin against some gram-positive organisms, especially staphylococci. The MIC of coumermycin for methicillin-susceptible or methicillin-resistant *Staphylococcus aureus* is usually lower than the MICs of the other antistaphylococcal antibiotics. Furthermore, in microdilution procedures, the MBC has been described as being very close to the MIC (1, 6, 9–11, 16, 17). In vivo, preliminary studies in mice suggest that coumermycin has a good protective activity against *S. aureus* infections (10, 15; J. Unowski, personal communication).

The newly developed fluoroquinolone compounds are active against *S. aureus* (19, 21), and since their bacterial target is the A subunit of DNA-gyrase (13, 22), it has been suggested and shown in vitro that these substances might act synergistically with coumermycin against gram-positive bacteria (17).

The purpose of the present study was to investigate in rats the activity of coumermycin and that of a fluoroquinolone, ciprofloxacin (1, 4, 5), in a model of left-sided endocarditis induced by *S. aureus*. This model of experimental endocarditis is a stringent test of antibiotic bactericidal activity, since the polymorphonuclear leukocytes have limited access to the bacteria embedded in the valvular vegetations (7). Because of the low therapeutic efficacy of coumermycin in endocarditis induced by one *S. aureus* strain, we further evaluated its activity in endocarditis induced by two additional strains of *S. aureus*. As we obtained similar results, we compared in vitro the susceptibilities to bacterial killing by coumermycin of these 3 strains with the susceptibilities of 12 additional strains of *S. aureus* isolated from patients with endocarditis.

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MATERIALS AND METHODS

Experimental strains. The 15 strains of *S. aureus* studied were isolated from the blood of patients with staphylococcal endocarditis.

One methicillin-susceptible strain of *S. aureus*, coded strain 1112, was used to induce endocarditis. This strain has been previously evaluated in this model (2). We examined the therapeutic efficacies of coumermycin, of ciprofloxacin, and of their combination and compared them with that of the cloxacillin-gentamicin combination that has exhibited the best efficacy against this strain in this model (2).

Two additional methicillin-susceptible strains of *S. aureus*, coded strain 7 and strain 560, were used to induce endocarditis to test further the therapeutic efficacy of coumermycin.

Twelve additional strains of *S. aureus* were used to test the in vitro susceptibility to coumermycin.

Susceptibility tests. For the 15 *S. aureus* strains, the MIC of coumermycin (Hoffmann-La Roche Inc.) was determined

* Corresponding author.

in Mueller-Hinton broth (Difco Laboratories) by the macrodilution method with an inoculum of 10^6 CFU/ml from an overnight culture (20). Coumermycin was solubilized in dimethyl sulfoxide before dilution in Mueller-Hinton broth. To determine the MBC, we plated 0.05-ml samples from each dilution of antibiotic showing no turbidity after 24 h of incubation onto Columbia blood agar (GIBCO Laboratories) with an automatic device (Spiral System DS; Interscience Laboratories, Saint-Nom, France). This device permitted us to diminish the antibiotic carry-over in that the sample was plated on a rotating agar plate, with a large volume expelled first near the center of the plate and decreasing logarithmically along the spiral path as it progressed toward the outside of the plate. Carry-over was further diminished by the addition of blood to the agar, since it has been described to partially inactivate coumermycin in vitro (6). The MBC was defined after 48 h of incubation as the lowest concentration of antibiotic which killed 99.9% of the inoculum.

For *S. aureus* 1112, the MICs and MBCs of ciprofloxacin (Bayer AG, Wuppertal, Federal Republic of Germany), cloxacillin (Beecham Laboratories), and gentamicin (Essex Chemie) were determined by the same methods (for the MBC determination of cloxacillin, turbidity in broth was recognized after 48 h of incubation, and penicillinase was added to the agar medium before subculturing for colony counts). For *S. aureus* 1112, *S. aureus* 7, and *S. aureus* 560, killing curves were determined in shaking flasks at 37°C with 5×10^5 CFU of log-phase bacteria per ml of tryptic soy broth (Difco) and 1 μg of coumermycin per ml, 5 μg of ciprofloxacin per ml, or the same concentration of each antibiotic in a coumermycin-ciprofloxacin combination. The concentration of each antibiotic was chosen so as to approximate the peak concentration of free antibiotic achieved in serum in treated animals.

Determination of the frequency of bacteria resistant to a coumermycin concentration 100-fold the MIC. For the 15 *S. aureus* strains, a concentrated bacterial suspension containing approximately 10^{10} CFU/ml was obtained by centrifugation of an overnight culture of each strain. Dilutions (10- and 100-fold) of this concentrate (containing 10^9 and 10^8 CFU/ml, respectively) were prepared by dilution in normal saline. A 0.1-ml sample from each bacterial suspension was plated onto Mueller-Hinton agar containing either no antibiotic or 0.4 μg of coumermycin per ml, a concentration 100-fold the MIC. The ratio of the number of colonies appearing on plates with and without antibiotic after 48 h of incubation was taken as an estimate of the frequency of bacteria resistant to a coumermycin concentration 100-fold the MIC (13). The results were expressed as the mean of three experiments.

Production of endocarditis. Sterile vegetations were produced in female Wistar rats (200 to 220 g) by insertion of a polyethylene catheter across the aortic valve through the right carotid artery as previously described (12). The catheter was secured with a silk ligature and left in place throughout the experiments. At 24 h after catheterization, rats were injected in the tail vein with 0.5 ml of a suspension prepared from an overnight culture of *S. aureus* to contain approximately 10^6 CFU/ml.

Since we have previously shown in this model of staphylococcal endocarditis that without antibiotic treatment, infection invariably leads to death of the rats within 3 days after bacterial challenge (2), no placebo-treated control rats were used in the present experiments. At the beginning of treatment (i.e., 24 h after bacterial challenge), rats were randomized either to be sacrificed to determine the incidence and the severity of infection (as measured by the CFU per gram of vegetations) or to receive 5 days of antibiotic treatment.

Treatment regimens. Rats infected with *S. aureus* 1112 and randomized for treatment received coumermycin at three different dosages, ciprofloxacin, the coumermycin-ciprofloxacin combination, or the reference treatment consisting of the cloxacillin-gentamicin combination. The three coumermycin dosage regimens studied were as follows: 4 mg/kg every 24 h (q 24 h), 4 mg/kg q 12 h, or 12 mg/kg q 12 h. Coumermycin was solubilized in a solvent (containing 11% alcohol [USP], 20% propylene glycol, 1% benzyl alcohol, and water [as much as suffices]) before further dilution in water and subcutaneous (s.c.) injection. The ciprofloxacin dosage regimen was 30 mg/kg q 8 h given s.c., either alone or in combination with coumermycin at a dosage of 4 mg/kg q 24 h. For the cloxacillin-gentamicin treatment, cloxacillin was injected s.c. at a dosage of 200 mg/kg q 8 h, and gentamicin was injected intramuscularly at a dosage of 4 mg/kg q 8 h.

Rats infected with *S. aureus* 7 or *S. aureus* 560 received only the high-dosage regimen of coumermycin (i.e., 12 mg/kg s.c. q 12 h). The therapeutic results were compared with those obtained with the same dosage regimen in endocarditis induced by strain 1112.

Evaluation of infection. The control rats were sacrificed 24 h after bacterial challenge. The treated rats were sacrificed after 5 days of antibiotic treatment (8 h after the last injections of ciprofloxacin and of cloxacillin-gentamicin and 24 h after the last injections of coumermycin and of coumermycin-ciprofloxacin. Blood (1 ml) was drawn from the inferior vena cava, plated onto Columbia blood agar, and incubated for colony counts. Aortic vegetations were excised aseptically, weighed, homogenized in 1 ml of saline, serially diluted, and plated onto Columbia blood agar. Colonies were counted after 24 h of incubation at 37°C, and the colony counts for each rat were expressed in $\log_{10}$ CFU per gram of vegetations. Rats that died during treatment were refrigerated at 4°C within 6 h of death; blood was not cultured from these animals, but their vegetations were processed as described above. Since death occurred at various times during treatment, individual bacterial densities of the vegetations of dying animals were not taken into account for comparison of the activity of the various antibiotic regimens.

Aliquots of vegetation homogenates from control and treated rats were routinely used for coumermycin and ciprofloxacin MIC determinations. The MICs were compared with the MIC for the bacteria contained in the inoculum used for challenge.

Antibiotic levels in serum in normal and infected rats. Antibiotic levels in serum were determined in normal rats at various intervals after the injection of a single dose of each antibiotic by either the enzyme multiplied immunoassay technique (EMIT; Syva Laboratories, Palo Alto, Calif.) for gentamicin levels or the agar diffusion technique (18) for coumermycin, ciprofloxacin, and cloxacillin levels. The assay organisms were *S. aureus* ATCC 29213 for coumermycin determinations, *Klebsiella pneumoniae* ATCC 10031 for ciprofloxacin determinations, and *Bacillus subtilis* (spore suspension; Difco) for cloxacillin determinations. The sensitivity of the techniques allowed us to assay concentrations as low as 0.5 μg/ml for gentamicin, 0.05 μg/ml for coumermycin, 0.2 μg/ml for ciprofloxacin, and 2 μg/ml for cloxacillin. In addition to antibiotic levels in serum in normal rats, peak and trough antibiotic levels in serum were determined by the same techniques in rats with endocarditis on

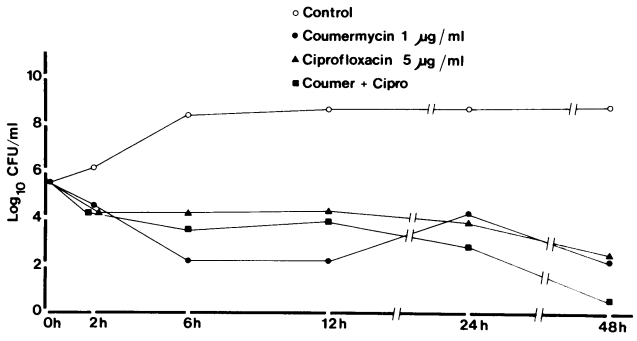

FIG. 1. Rates of in vitro killing of *S. aureus* 1112 with coumermycin (Coumer) and ciprofloxacin (Cipro), alone or in combination. The dosages used for the combination were the same as those used for the single-antibiotic experiments.

day 5 of treatment. The results were expressed as the mean of measures made in three different animals.

Statistical evaluation. The chi-square test with the Yates correction was used for the comparison of cumulative mortality and of sterilization of vegetations. The analysis of variance and the Student *t* test were used for the comparison of the bacterial counts in the vegetations.

RESULTS

Susceptibility tests of *S. aureus* 1112. The respective MIC and MBC of coumermycin for strain 1112 were <0.004 and 1 μg/ml, those of ciprofloxacin were 0.25 and 2 μg/ml, those of cloxacillin were 0.125 and 0.5 μg/ml, and those of gentamicin were 0.125 and 1 μg/ml. Thus, for strain 1112 the MBC/MIC ratio of coumermycin was high (equal to 250), but this was not the case with ciprofloxacin, cloxacillin, and gentamicin. The time-kill curves of strain 1112 in the presence of coumermycin and of ciprofloxacin are shown in Fig. 1. Coumermycin at a concentration as high as 250 times the MIC was bactericidal only during the first 6 h of incubation; ciprofloxacin at a concentration as high as 20 times the MIC was moderately bactericidal only during the first 2 h of incubation. The coumermycin-ciprofloxacin combination exhibited an increased activity over that observed with either single drug only after 48 h of incubation. For strain 1112, the frequency of bacteria resistant to a concentration of coumermycin 100-fold the MIC was 5×10^{-9}.

Susceptibility tests of *S. aureus* 7 and 560. For both strains, the MIC of coumermycin was <0.004 μg/ml, while the MBCs were 8 μg/ml for strain 7 and 4 μg/ml for strain 560; thus, the MBC/MIC ratios were 2,000 and 1,000, respectively. The time-kill curves with coumermycin were similar to those of strain 1112 (data not shown). The frequencies of bacteria resistant to a concentration of coumermycin 100-fold the MIC were 5×10^{-9} for strain 7 and 3×10^{-9} for strain 560.

Antibiotic levels in serum in normal rats. Levels of coumermycin and of ciprofloxacin in serum in normal rats are shown in Fig. 2. As described in humans (14), coumermycin had a long elimination half-life, approximately 12 h. For cloxacillin, 200 mg/kg s.c. resulted in concentrations in serum (± standard deviations) of 273 ± 69 μg/ml at 30 min and 11.7 ± 1.6 μg/ml at 2 h, while no detectable activity was found at 4 h. For gentamicin, 4 mg/kg intramuscularly resulted in concentrations in serum of 12.8 ± 4.3 μg/ml at 15 min and 2.3 ± 0.8 μg/ml at 2 h, while no detectable activity was found at 4 h.

Antibiotic levels in serum in rats with endocarditis. Peak and trough levels in serum measured on day 5 of endocarditis treatment are reported in Table 1. When compared with levels in serum in normal rats, there was some degree of accumulation of gentamicin and of coumermycin at the high-dosage regimen.

Efficacy of the various treatments against endocarditis induced by *S. aureus* 1112. The results of the different antibiotic regimens are reported in Table 2. The three coumermycin regimens were less effective than the cloxacillin-gentamicin combination in sterilizing the vegetations ($P < 0.05$) and in reducing the bacterial counts in the vegetations ($P < 10^{-8}$). In contrast, the ciprofloxacin treatment was as good as the cloxacillin-gentamicin treatment with respect to cumulative mortality, sterilization of the vegetations, and reduction of the bacterial counts in the vegetations. With regard to combined coumermycin-ciprofloxacin treatment, cumulative mortality and sterilization of the vegetations were not significantly different from those observed after ciprofloxacin monotherapy. However, the coumermycin-ciprofloxacin combination was less effective in reducing the bacterial counts than the cloxacillin-gentamicin combination ($P = 10^{-3}$) or ciprofloxacin alone ($P = 0.01$).

The MIC of coumermycin for the bacteria recovered from all the control rats was identical to the MIC for the original inoculum (<0.004 μg/ml), but the MIC of coumermycin for the bacteria recovered from 56 of 73 (77%) rats treated with coumermycin was increased, ranging from 0.008 to 2 μg/ml. Among these *S. aureus* variants less susceptible to coumermycin, the MIC for 46 of 56 (82%) rats ranged from 0.25 to 2 μg/ml (i.e., from 62 times the MIC to 500 times the MIC). The addition of ciprofloxacin to coumermycin for treatment did not prevent the emergence of such coumermycin-resistant variants, since the MIC of coumermycin for the bacteria recovered from all the rats treated with the coumermycin-ciprofloxacin combination was increased, ranging from 0.25 to 0.5 μg/ml (i.e., from 62 times the MIC to 124 times the MIC). The MIC of ciprofloxacin for the bacteria recovered from the rats treated with ciprofloxacin was unchanged.

Efficacy of the high-dosage regimen of coumermycin against endocarditis induced by *S. aureus* 7 and 560. The results of testing the high-dosage regimen of coumermycin against endocarditis induced by strains 7 and 560 are reported in Table 3 and are compared with the results of testing the same regimen against endocarditis induced by strain 1112. The cumulative mortality, the rate of sterilization of the vegetations, and the reduction of the bacterial counts in the

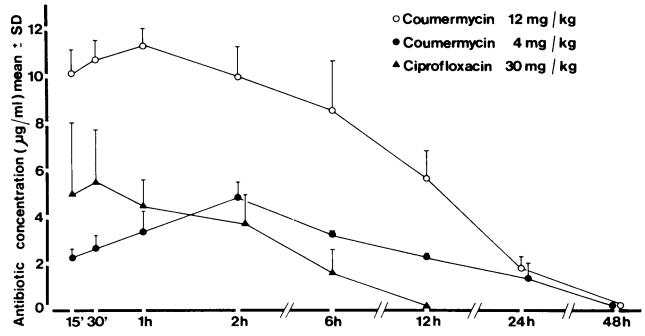

FIG. 2. Antibiotic levels in serum in normal rats after a single s.c. injection of coumermycin or ciprofloxacin. SD, Standard deviation.

TABLE 1. Antibiotic regimens and corresponding levels in serum measured in rats on day 5 of endocarditis treatment

Level being measured	Mean levels ± SD[a] of:					
	Coumermycin at:			Ciprofloxacin (30 mg/kg q 8 h)	Cloxacillin (200 mg/kg q 8 h)	Gentamicin (4 mg/kg q 8 h)
	4 mg/kg q 24 h	4 mg/kg q 12 h	12 mg/kg q 12 h			
Peak	5.4 ± 0.6 (2 h)	7.3 ± 0.4 (2 h)	23.9 ± 9.4 (2 h)	4.7 ± 1.3 (30 min)	260 ± 87 (30 min)	25.4 ± 13 (15 min)
Trough	1.2 ± 0.3 (24 h)	3.2 ± 0.5 (12 h)	13.2 ± 2 (12 h)	0.6 ± 0.04 (8 h)	ND[b]	0.8 ± 0.6 (8 h)

[a] Reported in micrograms per milliliter. Data in parentheses represent time of sampling after antibiotic injection.
[b] ND, Not determined.

vegetations were not significantly different for the three strains.

The MIC of coumermycin for the bacteria recovered from all the control rats was identical to the MIC for the original inocula (<0.004 μg/ml), but the MIC of coumermycin for the bacteria recovered from all the treated rats ranged from 0.25 to 8 μg/ml (i.e., from 62 times the MIC to 2,000 times the MIC).

Susceptibility tests of the 12 additional strains of *S. aureus*. Because coumermycin had a low therapeutic efficacy, we tested the susceptibility to killing of 12 additional strains of *S. aureus*, using precautions to avoid the carry-over of antibiotic. For all strains, the MIC of coumermycin was <0.004 μg/ml. The MBCs ranged from 0.032 to 8 μg/ml, the MBC for 50% of the strains being 4 μg/ml and the MBC for 90% of the strains being 8 μg/ml. For 1 strain, the MBC/MIC ratio was 8; for the 11 remaining strains, the MBC/MIC ratio ranged from 125 to 2,000. Thus, these results are in disagreement with those of other studies (1, 6, 9–11, 16, 17) which tested the MBC of coumermycin for *S. aureus* but did not consider the carry-over phenomenon. The frequency of bacteria resistant to a concentration of coumermycin 100-fold the MIC ranged from 2.7×10^{-9} to 3.1×10^{-8}.

DISCUSSION

In this experimental model of endocarditis, three coumermycin dosage regimens were far less effective than the cloxacillin-gentamicin combination in reducing the bacterial counts in the cardiac vegetations of rats infected with one strain of *S. aureus*. This low therapeutic efficacy of coumermycin was confirmed in endocarditis caused by two additional strains of *S. aureus*. In contrast to coumermycin, ciprofloxacin, which is also a DNA-gyrase inhibitor (8, 13, 22), was as effective as the cloxacillin-gentamicin combination in the treatment of endocarditis caused by one strain of *S. aureus*. These good therapeutic results of ciprofloxacin are in accordance with those obtained with another fluoroquinolone, pefloxacin, in the treatment of staphylococcal endocarditis in rabbits (19). The coumermycin-ciprofloxacin combination was less effective than ciprofloxacin alone in reducing the bacterial counts in the vegetations.

The low therapeutic efficacy of coumermycin in *S. aureus* endocarditis was observed despite coumermycin levels in serum 3-fold to 24-fold the MBC for the infecting strains at the peak and 1.6-fold to 13-fold the MBC at the trough on day 5 of the high-dosage regimen. This limited efficacy of coumermycin may have been due to several reasons. First, the determination of the MBCs for 15 *S. aureus* strains isolated from patients with staphylococcal endocarditis suggests that coumermycin has a limited bactericidal activity. Because of the extremely high growth-inhibiting activity of coumermycin on *S. aureus* (1, 6, 9–11, 16, 17), the determination of its bactericidal effect is difficult, since the antibiotic carry-over may impede the growth of surviving bacteria. This phenomenon leads to an underestimation of MBCs and an overestimation of the bactericidal effects of the drug in time-kill curves. Second, the selection during treatment of variants less susceptible or resistant to coumermycin may also be partly responsible for the therapeutic failure of coumermycin. The addition of ciprofloxacin to coumermycin

TABLE 2. Comparative efficacy of the various treatment regimens against endocarditis induced by *S. aureus* 1112

Treatment regimen	No. of rats that died during treatment/ total	No. of sterile vegetations/ total	Bacterial counts[a] in vegetations (mean ± SD)	No. of rats with positive blood cultures/ total
None[b]		0/33	9.47 ± 0.76	33/33
Cloxacillin + gentamicin	7/26	8/26	4.65 ± 1.04[c]	0/19[c]
Coumermycin (4 mg/kg q 24 h)	21/29	2/29	9.45 ± 1.04[c]	6/8[c]
Coumermycin (4 mg/kg q 12 h)	12/21	0/21	8.94 ± 0.75[c]	3/9[c]
Coumermycin (12 mg/kg q 12 h)	12/23	0/23	8.41 ± 1.33[c]	1/11[c]
Ciprofloxacin	6/26	5/26	5.32 ± 1.54[c]	0/20[c]
Coumermycin (4 mg/kg q 24 h) + ciprofloxacin	6/38	4/38	6.65 ± 1.88[c]	0/32[c]

[a] Expressed in $\log_{10}$ CFU per gram of culture-positive vegetations.
[b] Control rats were sacrificed 24 h after bacterial challenge at the beginning of treatment.
[c] Data were derived only from rats that survived the 5-day treatment.

TABLE 3. Comparative efficacy of the high-dosage regimen of coumermycin against endocarditis induced by the reference *S. aureus* strain (1112) and by two additional *S. aureus* strains (strains 7 and 560)

Treatment regimen	*S. aureus* strain	No. of rats that died during treatment/ total	No. of sterile vegetations/ total	Bacterial counts[a] in vegetations (mean ± SD)	No. of rats with positive blood cultures/ total
None[b]	1112		0/33	9.47 ± 0.76	33/33
	7		0/14	9.23 ± 1.10	14/14
	560		0/13	9.05 ± 1.01	13/13
Coumermycin (12 mg/kg q 12 h)	1112	12/23	0/23	8.41 ± 1.33[c]	1/11[c]
	7	14/25	2/25	8.18 ± 0.98[c]	0/11[c]
	560	15/21	0/21	8.56 ± 0.87[c]	1/6[c]

[a] Expressed in $\log_{10}$ CFU per gram of culture-positive vegetations.
[b] Control rats were sacrificed 24 h after bacterial challenge at the beginning of treatment.
[c] Data were derived only from rats that survived the 5-day treatment.

in the treatment did not prevent the emergence of such coumermycin-resistant variants. Mutants less susceptible to coumermycin could be selected in vitro from all 15 *S. aureus* strains tested. Third, it has been found in vitro that coumermycin may be inactivated by serum or blood (6, 15), but the relevance of this phenomenon has not been studied so far. It is conceivable that it may have accounted somewhat for the limited efficacy observed in the present experiments. Fourth, the penetration of coumermycin into cardiac vegetations is unknown, but because the drug is a large molecule with a molecular weight of 1,106 (13), it might be low.

The therapeutic failure of coumermycin in our model of staphylococcal endocarditis is in contrast to the good protective activity of coumermycin already described in models of thigh or peritoneum staphylococcal infections in mice (10, 15; J. Unowski, personal communication). Since coumermycin was injected at the time of bacterial challenge in these protection models, the bacterial load at the start of treatment was considerably lower than after 24 h of systemic infection, as in our endocarditis model.

The good therapeutic efficacy of ciprofloxacin in *S. aureus* endocarditis was observed at ciprofloxacin levels in serum 2.3-fold the MBC for the infecting strain at the peak and 0.3-fold the MBC at the trough. The susceptibility of the bacteria to ciprofloxacin was not modified during treatment.

In conclusion, coumermycin, despite its very high bacteriostatic activity in vitro, was disappointing in the treatment of experimental *S. aureus* endocarditis. This model requires a bactericidal activity of the therapeutic agent because the bacterial load is high and because the catheter is left in place throughout the experiments, mimicking prosthetic valve endocarditis. Ciprofloxacin exhibited a high antistaphylococcal activity in the same model, but its combination with coumermycin reduced its therapeutic efficacy. Coumermycin will not be developed further for clinical use (M. Fernex, personal communication).

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