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Ceftriaxone-Sulbactam Combination in Rabbit Endocarditis Caused by a Strain of *Klebsiella pneumoniae* Producing Extended-Broad-Spectrum TEM-3 β-Lactamase

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We studied the activity of the combination of sulbactam and ceftriaxone against a *Klebsiella pneumoniae* strain producing TEM-3, a new extended-broad-spectrum β-lactamase, in an endocarditis model. In vitro, ceftriaxone was strongly inactivated in the presence of TEM-3 (MBC, 128 μg/ml with an inoculum of 5×10^5 CFU/ml). A marked inoculum effect was demonstrated with sulbactam: effective concentrations of inhibitor needed to reduce the MIC and MBC of ceftriaxone to similar levels increased from 1 μg/ml in the presence of an inoculum of 5×10^5 CFU/ml to 20 μg/ml in the presence of an inoculum of 1×10^7 CFU/ml. In vivo, sulbactam given at 200 mg/kg of body weight every 12 h, a dosage higher than that previously reported to be effective against rabbit endocarditis caused by other microorganisms, was not sufficient to restore the complete activity of ceftriaxone given at 30 mg/kg once daily for 4 days. This insufficient activity may be correlated with the presence of a high level of β-lactamase inside the vegetations, as indicated by a quantitative in vitro assay of β-lactamase activity in the cardiac vegetation, suggesting an insufficient inactivation of the extended-broad-spectrum β-lactamase in vivo.

Infections caused by multiresistant pathogens are frequently epidemic and represent a major therapeutic challenge. Drug combinations that include an irreversible β-lactamase inhibitor may be a novel approach to the treatment of those infections caused by β-lactamase-producing strains (4).

TEM-3, previously designated CTX-1, is a new constitutively plasmid-mediated extended-broad-spectrum β-lactamase that has recently been described among members of the family *Enterobacteriaceae* and that was found to be responsible for nosocomial outbreaks in several hospitals in France (3, 13, 23). In vitro, TEM-3-producing *Klebsiella pneumoniae* is resistant to all β-lactams except cephamycins and imipenem (3, 23). Irreversible β-lactamase inhibitors such as clavulanic acid and sulbactam reduce the MICs of hydrolyzed β-lactams to normal and seem to be interesting alternatives for the therapy of infections caused by extended-broad-spectrum β-lactamase-producing strains (13, 15). However, the combination of a β-lactam antibiotic with an inhibitor of β-lactamase must be evaluated in animal models before clinical investigations can be performed.

The aim of this study was to evaluate in vivo the conditions under which sulbactam, an irreversible β-lactamase inhibitor, is able (or not able) to restore the activity of ceftriaxone, a broad-spectrum cephalosporin hydrolyzed by TEM-3. The activity of sulbactam in combination with ceftriaxone against a strain of *K. pneumoniae* that produces a TEM-3 which hydrolyzes ceftriaxone was compared with the activity of ceftriaxone alone against a *K. pneumoniae* variant that does not produce TEM-3 and that is susceptible to ceftriaxone. The in vivo study was performed by using an experimental endocarditis model that provides a rigorous test for studying the efficacy of antimicrobial agents against severe infections with high local concentrations of bacteria (5). The results obtained in vivo with the sulbactam-ceftriaxone combination were analyzed according to the pharmacokinetic parameters of sulbactam, the in vitro activity of sulbactam, and the activity of sulbactam against the β-lactamase in vitro as well as in cardiac vegetations.

MATERIALS AND METHODS

Bacterial strains. *K. pneumoniae* CF104 was isolated from the blood of a septicemic patient. This strain harbors a self-transferable plasmid, pCF104, that encodes for only one β-lactamase (TEM-3), an aminoglycoside acetyltransferase 6′IV, and resistance to tetracyclines and sulfonamides (23). Its susceptible variant that was spontaneously obtained in vitro had lost these resistance markers. Both strains produced the common chromosomal SHV-1 β-lactamase found in *K. pneumoniae*, although they produced it at a very low level.

The resistant and susceptible *K. pneumoniae* strains were resistant to rabbit serum and were able to reliably induce an experimental endocarditis in these animals.

Antibiotics. Ceftriaxone was supplied by Roche Laboratories (Neuilly, France), and sulbactam was supplied by Pfizer Laboratories (Orsay, France).

Susceptibility testing. MICs and MBCs were determined by the tube macrodilution method in Mueller-Hinton broth supplemented with Ca^{2+} and Mg^{2+} (Diagnostic Pasteur, France) (22). Each value was determined with a culture that was in the logarithmic phase of growth at final inocula of 5×10^5 and 1×10^7 CFU/ml in order to test susceptibilities under standard conditions and to reproduce in vitro the high bacterial concentrations observed in cardiac vegetations. The MIC was defined as the lowest concentration of drug that inhibited growth, i.e., visible turbidity after 24 h of

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incubation at 37°C. The MBC was determined by subculturing 0.01 ml from each clear tube onto agar plates and was defined as the lowest concentration that reduced the number of viable organisms by 99.9%. MICs and MBCs were determined for sulbactam alone and for ceftriaxone either alone or combined with a fixed concentration of sulbactam (1, 10, or 20 μg/ml).

Pharmacokinetics of sulbactam. Serum samples were collected from four noninfected female New Zealand White rabbits at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 h after a single intramuscular (i.m.) injection of 200 mg of sulbactam per kg of body weight. In eight animals with experimental endocarditis infected with the resistant strain of *K. pneumoniae*, cardiac vegetations were collected either 1 h ($n = 2$) or 12 h ($n = 6$) after the eighth i.m. injection of 200 mg of sulbactam given twice daily (BID). Serum and vegetations were stored at −80°C until the assays were performed. The concentrations of sulbactam in sera and vegetations were determined by high-pressure liquid chromatography by the technique described by Rogers et al. (20), with minor modifications. The limit of sensitivity was 0.1 μg/ml.

Pharmacokinetics of ceftriaxone. In this study, previously described data (12, 17) on the pharmacokinetics of ceftriaxone were used under the same experimental conditions.

Experimental endocarditis. Bacterial aortic endocarditis was established by the technique by Perlman and Freedman (18), as follows. On day 1, rabbits were anesthetized by a ketamine hydrochloride i.m. (15 mg/kg). The right carotid artery was exposed and cannulated with a polyethylene catheter. The catheter was advanced until pulsations and resistance indicated that it had reached the apex of the left ventricle. It was then sealed with a ligature and left in place for the duration of the experiment. Twenty-four hours after surgery, animals were injected in the marginal ear vein with approximately 10^9 CFU (under a volume of 1 ml) of *K. pneumoniae* (resistant or susceptible strain). Only animals with both weight loss and at least one of three positive blood cultures on day 4 were included in the study. Treatment was performed from days 5 to 8 (i.e., for 4 days). Ninety-two rabbits were randomly assigned to one of the following eight groups: untreated controls (resistant and susceptible strains), ceftriaxone given at 30 mg/kg i.m. once daily (OD) (resistant and susceptible strains), sulbactam given at 200 mg/kg i.m. BID (resistant strain), sulbactam given at 200 mg/kg i.m. every 6 h (QID) (resistant strain), ceftriaxone given OD plus sulbactam given BID (resistant strain), and ceftriaxone given OD plus sulbactam given QID (resistant strain). On day 9, animals were killed at the time of measurement of the residual concentration of each drug, i.e., 24 h after the last injection of ceftriaxone given OD, 12 h after sulbactam given BID, or 6 h after sulbactam given QID. The heart was removed and inspected; only animals in which the catheter was correctly placed across the aortic valve were included in the study. All vegetations from each animal were excised, pooled, weighed, and rinsed in sterile saline. Vegetations were homogenized in 0.5 ml of sterile saline, and 0.1-ml portions were quantitatively subcultured onto agar plates at 37°C for 24 h. The lower limit of detection by this method was 2 $\log_{10}$ CFU/g. If the titer was below this value, the vegetation was considered sterile. The corresponding titer was considered to be 2 in calculating the means. Data from animals which died before completion of therapy were included only if the duration of treatment was sufficient, i.e., 3 full days (24). Means of bacterial titers were compared by an analysis of variance followed by comparison of means by the Bonferroni method (11).

TABLE 1. MICs and MBCs of ceftriaxone with and without sulbactam against two strains of *K. pneumoniae* that produced or did not produce the TEM-3 extended-broad-spectrum β-lactamase at two different inocula

K. pneumoniae strain and antibiotic	5×10^5 CFU/ml		1×10^7 CFU/ml	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
Susceptible (nonproducing)				
Ceftriaxone	0.25	0.25	0.25	0.50
Sulbactam	32	32	32	32
Resistant (producing)				
Ceftriaxone	32	128	>1,000	>1,000
Sulbactam	32	32	32	32
Ceftriaxone + sulbactam (1 μg/ml)	0.25	0.25	4	16
Ceftriaxone + sulbactam (10 μg/ml)	0.12	0.25	0.25	1
Ceftriaxone + sulbactam (20 μg/ml)	ND[a]	ND	0.12	0.50

[a] ND, Not done.

Ex vivo β-lactamase assays. Eighteen cardiac vegetations were collected on day 9 from six untreated rabbits infected with the resistant *K. pneumoniae* strain. For each vegetation, four assays of β-lactamase activity were successively performed as described above. Immediately after sacrifice, the first assay was performed on the intact vegetation. Then, the vegetation was incubated in phosphate buffer (10 mM, pH 7) with various concentrations of sulbactam (0, 1, 10, or 100 mg/liter) at 37°C during 30 min before the second assay. For the other two assays, the same vegetation previously exposed to sulbactam was used. It was washed twice in phosphate buffer, suspended in 1.5 ml of the same buffer, homogenized, and sonicated to release all the residual β-lactamase activity from the bacteria contained in the vegetation. After centrifugation, the supernatant was divided into two samples. The first sample (third assay) was used to determine the residual β-lactamase activity. The second sample (fourth assay) was used to examine the effect of sulbactam on the crude extract of β-lactamase and was first incubated for 30 min with a concentration of sulbactam similar to that used with the corresponding intact vegetation. For each assay the β-lactamase activity was determined spectrophotometrically at 30°C with a double-beam spectrophotometer coupled to a recorder (models 550 S and 561; Perkin-Elmer Corp.) by using nitrocefin (100 μM) as the substrate. One unit of activity was defined as 1 nmol of nitrocefin hydrolyzed per min/g of vegetation.

RESULTS

Susceptibility testing. MBCs and MICs are presented in Table 1. The concentrations of sulbactam that were able to lower the MBCs of ceftriaxone for the resistant strain to the values measured for the susceptible strain were 1 and 20 μg/ml for the inocula of 5×10^5 and 1×10^7 CFU/ml, respectively.

Pharmacokinetics of sulbactam. Concentrations of sulbactam in the sera of rabbits are reported in Table 2. A monoexponential decline with a half-life of 1.3 h in serum was noted. Sulbactam levels in vegetations were 98.8 and 89.3 μg/g for the two samples tested 1 h after the last injection but were not detectable at 12 h.

TABLE 2. Sulbactam concentrations in serum of noninfected rabbits after a single i.m. injection of 200 mg of sulbactam per kg

Time (h)	Concn in serum (μg/ml)
0.25	235.2 ± 55.2
0.5	256.4 ± 69.2
0.75	251.7 ± 52.8
1	213.2 ± 40
1.5	135.1 ± 28.3
2	96.5 ± 23.8
3	50.2 ± 13.2
4	30.5 ± 12.1
6	10.7 ± 4.5
8	5.9 ± 2.7
12	0

Pharmacokinetics of ceftriaxone. Ceftriaxone levels previously reported in the serum of New Zealand White rabbits (12, 17) following a single i.m. injection of 30 mg/kg were 72 ± 8.6 μg/ml at 1 h and 0.4 ± 0.3 μg/ml at 24 h. The elimination half-life in serum was 2.8 ± 0.45 h. Levels in infected vegetations were 28 ± 16, 1.7 ± 1.2, and 0.8 ± 0.5 μg/g at 2, 12, and 24 h, respectively.

Experimental endocarditis. In vivo results are given in Table 3. In the susceptible *K. pneumoniae* group, ceftriaxone alone significantly reduced the bacterial counts ($P < 0.01$) compared with that in the control group. In the resistant *K. pneumoniae* groups, ceftriaxone plus sulbactam given BID did not significantly reduce the bacterial counts. Sulbactam given QID either alone or in combination with ceftriaxone was able to reduce significantly the CFU per gram of vegetation ($P < 0.01$ versus control in each group) compared with that in the control group.

Ex vivo β-lactamase assays. β-Lactamase activities in intact or sonified vegetations before or after incubation with sulbactam are given in Table 4. Homogenization and sonication released β-lactamase activity from the bacteria that were present in the cardiac vegetations; hydrolysis rates increased from 64 to 596 mU (ratio, 9.3) in the control group. When the intact vegetations were preexposed to different concentrations of sulbactam without sonication, a 75 to 90% reduction of β-lactamase activity was obtained. However, after washing and sonication of the same vegetations, no decrease in the total β-lactamase activity was observed compared with that in controls. The concentration of sulbactam needed to inhibit totally the β-lactamase activity after sonication was between 1 and 10 μg/ml.

TABLE 4. β-Lactamase activity assayed in rabbit cardiac vegetations infected by *K. pneumoniae* producing the TEM-3 extended-broad-spectrum β-lactamase

No. of vegetations	Sulbactam concn (μg/ml)[a]	β-Lactamase activity (mU) in[b]:			
		Assay 1	Assay 2	Assay 3	Assay 4
6	0	64 ± 31	ND[c]	596 ± 533	ND
4	1	95 ± 50	24 ± 6.6	529 ± 346	103 ± 77
3	10	49 ± 13	12.6 ± 7.0	701 ± 600	0
5	100	60 ± 52	6.6 ± 3.0	566 ± 407	0

[a] Concentration of sulbactam used in the assays.
[b] One unit = 1 nmol of nitrocefin hydrolyzed per min/g of vegetation. β-Lactamase activity was evaluated for each vegetation in four consecutive assays: assay 1, on intact vegetation without sulbactam; assay 2, same vegetation as in assay 1 after exposure to sulbactam for 30 min; assay 3, same vegetation as in assay 1 after washing, homogenization, and sonication; assay 4, same sonified extract as in assay 3 after exposure to sulbactam (same concentration as in assay 1) for 30 min. Values are means ± standard deviations and represent the mean β-lactamase activity determined in the indicated number of vegetations.
[c] ND, Not done.

DISCUSSION

The two strains of *K. pneumoniae* used in this study were absolutely identical except for the presence in the resistant strain of a plasmid-mediated β-lactamase, TEM-3. Thus, the differences in antibiotic activities noted in vitro and in vivo between the resistant and susceptible strains could be attributed exclusively to the presence of the TEM-3 β-lactamase.

In vitro, the MIC and MBC of ceftriaxone for the susceptible strain (0.25 μg/ml at 5×10^5 CFU/ml) were within the range of those reported previously for susceptible strains (16). The antimicrobial activity of sulbactam alone against both resistant and susceptible strains was poor (MIC and MBC, 32 μg/ml at inocula of 5×10^5 and 1×10^7 CFU/ml), as reported previously with other members of the family *Enterobacteriaceae* (7, 19). A high inoculum did not increase the MIC and MBC of ceftriaxone for the susceptible strain or those of sulbactam for both the resistant and susceptible strains. In contrast, the MIC of ceftriaxone for the resistant strain increased from 32 μg/ml with the lowest inoculum to more than 1,000 μg/ml with the highest inoculum. As reported previously (13, 15), sulbactam is able, in vitro, to restore completely the activity of a broad-spectrum cephalosporin like ceftriaxone which is hydrolyzed by the TEM-3 β-lactamase. However, the effective concentrations of β-lactamase inhibitor needed to reduce the MBC of ceftriaxone for the resistant strain to a level similar to that found for the

TABLE 3. Results of different 4-day therapeutic regimens in rabbits with endocarditis caused by *K. pneumoniae* variants that produced or did not produce the TEM-3 broad-extended-spectrum β-lactamase

Antibiotic regimen (single dose, mg/kg)	Resistant (producing) variant		Susceptible (nonproducing) variant	
	Log_{10} CFU/g of vegetation (mean ± SD)	No. of sterile vegetations/ total no. (%)	Log_{10} CFU/g of vegetation (mean ± SD)	No. of sterile vegetations/ total no. (%)
Control	7.0 ± 1.8	0/11 (0)	8.1 ± 1.6	0/7 (0)
Ceftriaxone (30) OD	6.9 ± 1.8	0/9 (0)	4.9 ± 2.3[a]	2/8 (25)
Sulbactam (200) BID	6.6 ± 2.2	1/9 (11)	ND[b]	ND
Sulbactam (200) QID	4.1 ± 1.7[a]	3/6 (50)	ND	ND
Ceftriaxone (30) OD + sulbactam (200) BID	5.4 ± 2.3	2/9 (22)	ND	ND
Ceftriaxone (30) BID + sulbactam (200) QID	4.1 ± 2.0[a]	2/5 (40)	ND	ND

[a] Significantly different from control values ($P < 0.01$).
[b] ND, Not done.

susceptible strain increased from 1 μg/ml at an inoculum of 5×10^5 CFU/ml to 20 μg/ml at an inoculum of 1×10^7 CFU/ml, demonstrating a marked in vitro inoculum effect. At the high inoculum, the concentration of sulbactam needed to significantly decrease the MBC of ceftriaxone (20 μg/ml) was close to the MIC and MBC (32 μg/ml) of sulbactam alone for the two strains.

The pharmacokinetic profile of sulbactam in serum appeared to be monoexponential in rabbits, with a half-life in serum of 1.3 h; this result was approximately similar to that observed in healthy humans (10). One hour after injection, sulbactam concentrations in vegetations reached approximately half of the levels in serum. This ratio (1:2) was similar to that observed with other β-lactams, such as ceftriaxone (12, 17). Twelve hours after injection, sulbactam was undetectable in vegetations; elimination of the drug appeared to be similar in vegetations and sera. These data are in agreement with those of a previous study (5), in which it was demonstrated that cardiac vegetation can be considered a compartment of rapid exchange with plasma without any accumulation.

An antibiotic carry-over within the vegetation that would have been able to modify the evaluation of in vivo results was eliminated, since 10^{-2} to 10^{-4} dilutions of homogenized vegetations were used to determine the bacterial titers. In vivo, bacterial titers in resistant and susceptible control groups were not significantly different in animals infected with the same inoculum, suggesting that both strains had similar degrees of virulence. Ceftriaxone alone was active against the susceptible strain, as indicated by a significant decrease in the bacterial titers (−3.2 log units versus the controls; $P < 0.01$), and was ineffective against the resistant strain. These results are in agreement with the bacteriological and pharmacokinetic data. The ceftriaxone concentration/MBC ratio in vegetation was low for the resistant strain (ratio, 0.22). Previous studies demonstrated that the local antibiotic peak level/MBC ratio necessary to obtain a bactericidal effect of several cephalosporins (including ceftriaxone) in an *Escherichia coli* endocarditis model should be greater than 100 to maintain concentrations greater than the MIC over the total interval between doses (12, 17). The same argument may explain why only a poor rate of sterilization (25%) was obtained with the ceftriaxone regimen against susceptible strains which presented a relatively high MIC and MBC of ceftriaxone (0.25 and 0.5 μg/ml) for a susceptible strain.

The regimen that delivered 200 mg of sulbactam per kg i.m. every 12 h was chosen because it yielded a trough level of 1 μg/ml in serum, a value that was able to restore the in vitro activity of ceftriaxone at an inoculum of 5×10^5 CFU/ml. Given alone, sulbactam was ineffective, which is in agreement with the MICs and MBCs. When sulbactam was given BID with ceftriaxone (30 mg/kg OD), it was not sufficient to restore the in vivo activity of ceftriaxone, as indicated by the nonsignificant decrease in bacterial titers (−1.6 log units versus the control). This insufficient activity cannot be explained by an inadequate dosage of ceftriaxone since with the same regimen (30 mg/kg OD), ceftriaxone alone was active against the susceptible strain. The dosage of sulbactam used was higher than that previously reported to be active in combination with ampicillin or cefoperazone in several experimental models of endocarditis caused by penicillinase-producing *Staphylococcus aureus* (7, 25; A. S. Bayer, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 369, 1988; C. Thauvin-Eliopoulos, L. B. Rice, G. M. Eliopoulos, and R. C. Moellering, Jr., 28th ICAAC, abstr. no. 367, 1988). It was also higher than that which is effective in combination with ceftriaxone against an SHV-2-producing *E. coli* strain that causes experimental endocarditis (9). A lack of penetration of sulbactam into cardiac vegetations was excluded by the high concentration of the inhibitor observed in the cardiac vegetation 1 h after the i.m. injection and by the efficacy of the inhibitor in the models of endocarditis mentioned above. However, a heterogeneous distribution of sulbactam into vegetation as described with other drugs by an autoradiographic method cannot be excluded (6). A lack of penetration of sulbactam into the bacteria was also unlikely since in vitro, at a low inoculum, the activity of ceftriaxone could be restored with only 1 μg of sulbactam per ml. The high degree of binding of ceftriaxone to proteins in serum might have contributed to the lower-than-expected efficacy of the ceftriaxone-sulbactam combination against resistant strains. In fact, a fourfold increase in MICs and MBCs of ceftriaxone against various strains of *K. pneumoniae* was observed by Leggett and Craig (14) in vitro in the presence of 95% serum. However, the in vivo significance of this phenomenon has not been established because of the concentration dependence of the binding of ceftriaxone to serum proteins. In our study, the efficacy of ceftriaxone alone against the susceptible strain, a strain which differs from the resistant strain only by the absence of the TEM-3 enzyme, suggests that the effect of serum on the in vivo activity of this antibiotic was probably poor with the dosage regimen used.

One possible explanation could be the presence of a high density of bacteria inside the vegetation. Histological studies showed clusters of bacteria inside the cardiac vegetations (unpublished data). This could result in the local production of very high levels of β-lactamase and in the greater heterogeneity of this activity from one vegetation to another, as shown in Table 4. Some in vitro and ex vivo results would favor this hypothesis. With a high inoculum in vitro, high concentrations of sulbactam (which were never reached at the trough level with sulbactam given BID) were necessary to decrease the MIC and MBC of ceftriaxone to a level compatible with in vivo efficacy. In the ex vivo study, even after 30 min of preexposure to very high concentrations of inhibitor, the apparent absence of irreversible inactivation of the β-lactamase would suggest that an insufficient concentration of inhibitor was present in relation to the high quantity of β-lactamase (21). Therefore, it is interesting to try to understand the discrepancy between the weak activity of the sulbactam-ceftriaxone combination obtained in this model and the success of the same treatment in an endocarditis model caused by an *E. coli* producing the extended-broad-spectrum β-lactamase SHV-2 (9). Quantitative examination after isoelectric focusing of the SHV-2 and TEM-3 β-lactamases produced by the strains used in endocarditis models described here showed that production of TEM-3 by the resistant strain was at least eightfold higher than production of SHV-2 by *E. coli* (L. Gutmann, unpublished data) and correlated well with the MICs observed at low (5×10^5 CFU/ml) and high (1×10^7 CFU/ml) inocula, which were 2 and 256 μg/ml, respectively, for *E. coli* SHV-2 and 32 and >1,000 μg/ml, respectively, for *K. pneumoniae* CF104.

Interestingly, it was previously reported for a model of rabbit endocarditis caused by a *Pseudomonas aeruginosa* strain that constitutively overproduced a cephalosporinase that the low efficacy of a combination of ceftazidime and dicloxacillin (used as a reversible inhibitor of the cephalosporinase) was due to the high density of bacteria present in the vegetation (2). A synergy between the β-lactam (ceftazi-

dime) and the reversible β-lactamase inhibitor (dicloxacillin) was observed in vitro only at a low inoculum (10^5 CFU/ml), and it was suggested that at a high inoculum (10^7 CFU/ml), dicloxacillin was slowly hydrolyzed or rendered incapable of rebinding the enzyme (2).

Finally, an unexpected result was the in vivo efficacy of sulbactam given QID alone (200 mg/kg i.m. every 6 h). This very high dosage regimen is inconceivable in humans because of the risk of adverse reactions. However, in the present study, it allowed us to show that when levels in serum were very high (always higher than 20 mg/liter for 4 days), sulbactam was active both alone and in combination with ceftriaxone. These data suggest that the antimicrobial activity of the β-lactamase inhibitor alone is sufficient in vivo to reduce significantly the bacterial counts but is inadequate to sterilize the vegetations.

Results of this study demonstrated an inoculum effect in vitro of a strain of *K. pneumoniae* producing an extended-broad-spectrum β-lactamase such as TEM-3 on sulbactam and suggest that the high concentration of bacteria in cardiac vegetations and the associated high rate of production of TEM-3 β-lactamase may be the explanation for the poor activity of the sulbactam-ceftriaxone combination observed in vivo.

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