Evidence of Less Severe Aortic Valve Destruction after Treatment of Experimental Staphylococcal Endocarditis with Vancomycin and Dexamethasone

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The beneficial effects of therapy combining an antibiotic and dexamethasone have been reported in human studies on meningitis and in experimental studies on septic arthritis, nephritis, and endophthalmitis. Since most patients with staphylococcal endocarditis need a combination of medical and surgical treatment, the purpose of this study was to determine whether the addition of dexamethasone to vancomycin has any beneficial effect regarding the degree of valve tissue damage or the course of experimental aortic valve endocarditis caused by a methicillin-resistant strain of Staphylococcus aureus. Rabbits with catheter-induced aortic valve vegetations were randomly assigned to a control group and to groups receiving dexamethasone (0.5 mg/kg of body weight, intravenously [i.v.], twice a day [b.i.d]), vancomycin (30 mg/kg, i.v., b.i.d), or dexamethasone plus vancomycin, for a total of 10 doses (two doses per day for 5 days). The severity of valve tissue damage was significantly less in groups receiving vancomycin plus dexamethasone compared with that of the group receiving vancomycin alone (P < 0.001). The severity of tissue damage was inversely correlated with the mean polymorphonuclear leukocyte number in valve tissue. No statistically significant differences were observed between the vancomycin-treated group and the vancomycin-plus-dexamethasone-treated group in survival, blood culture sterilization rate, or reduction of the microbial burden (in CFU per gram) in valvular tissue. In conclusion, treatment with a combination of vancomycin and dexamethasone for 5 days reduces the severity of valve tissue damage in experimental staphylococcal aortic valve endocarditis. These findings could have significant implications in the treatment of staphylococcal endocarditis and deserve further confirmation in clinical trials.

Staphylococcus aureus is the second most common cause of infective endocarditis (IE), affecting both native (25 to 35%) and prosthetic (33) valves, and is characterized by valve destruction and significantly higher in-hospital mortality. In some medical centers, staphylococcal endocarditis may even predominate (23). S. aureus IE may occur in patients of any age who are apparently healthy and in intravascular device users, who are usually young. Patients with community-acquired staphylococcal bacteremia are at high risk for IE. However, the recent increases in the frequencies of intravascular-device-associated and nosocomial staphylococcal bacteremias and even of staphylococcal bacteremia complicating outpatient parenteral antimicrobial therapy are some possible explanations for the increase in the number of patients at risk for IE (6). During the course of staphylococcal endocarditis, most patients will eventually need valve replacement (32). Early combined medical and surgical intervention is more beneficial than medical therapy alone in both native-and prosthetic-valve IE; thus, a more aggressive surgical approach has been recommended during the last 10 years (12, 21, 32).

Dexamethasone has been successfully used together with an antibiotic in the treatment of experimental staphylococcal endocarditis caused by a methicillin-resistant strain of Staphylococcus aureus (MRSA), the effect of the addition of dexamethasone to the therapeutic regimen. To our knowledge, no similar study has yet been published.

This research was presented previously (P. Siaperas et al., Abstr. 38th Annu. Meet. Infect. Dis. Soc. Am., abstr. 34, 2000).

MATERIALS AND METHODS

Bacterial strain. The endocarditis-inducing strain of S. aureus (SA-443) used in the present study was a clinical isolate obtained from a patient with sepsis. The
bacteria were stored at ~80°C in skim milk and were subcultured on blood agar plates 5 days before removal for experiment.

Susceptibility testing and time-kill curves. The MICs and minimal bactericidal concentrations (MBCs) of oxacillin (plus 2% NaCl) and vancomycin were determined by a microdilution technique in Mueller-Hinton broth (BBL Microbiology Systems) with the concentrations of the antibiotics tested ranging from 0.25 to 512 μg/ml. The MICs of the antibiotics were determined with inocula of ~5 × 10^6 and ~5 × 10^7 CFU/ml because the latter number of bacteria simulates more closely the pretreatment bacterial load in the vegetations. The MBC was determined by subculturing 0.1 ml from each well clear to antibiotic-free blood agar plates and was defined as the lowest concentration that reduced the number of organisms of the initial inoculum by ≥99.9%.

The in vitro bactericidal effect of vancomycin was assessed by the time-kill curve method. An overnight culture in Mueller-Hinton broth was used to prepare inocula of ~5 × 10^6 and ~5 × 10^7 CFU/ml. The final antibiotic concentration tested was equivalent to the MBC, as determined with an inoculum of ~5 × 10^6 CFU/ml, of vancomycin (30 mg/kg every 12 h, intravenously) on the sterilization (absence of dexamethasone (1 mg/kg of body weight every 12 h, intravenously) plus inflammation).

Observation of the aortic valves under light microscopy (presence of bacteria and evidence of inflammation). An overnight culture in Mueller-Hinton broth was used to pre-

Quantitative peripheral blood samples. Blood samples (~1 ml) for quantitative culture were obtained from the ear artery 1 day after inoculation of the bacterial strain, just before the administration of the first dose of antibiotic (day 2), and on days 4 and 6 (day of sacrifice). To day 6, blood was taken from the left ventricle after a small dose of heparin was injected into the sacrificed animal.

Histopathological studies. Tissue specimens were fixed immediately in 10% neutral buffered formalin for 24 h and embedded in paraffin. Five-micrometer-thick sections were cut and stained with hematoxylin and eosin and Giemsa stain for bacterial colonies and Masson-trichrome stain for delination of vegetations. The histological findings were read blindly by the pathologist (A. Kyroudi-Voulgaris), without any information regarding either treatments or the results of the valve cultures. The absolute number of neutrophils (polymorphonuclear leukocytes [PMNs]) was evaluated in 10 random optical fields of the periphery of the vegetation on the side of the valve under high-power magnification. PMNs were counted by use of a 100-point double square grid incorporated in the eye lens at a final magnification of ×400. The histopathological findings for the valvular tissue damage were classified into three categories, mild, moderate, and severe. This classification was based on the presence of fibrosis as well as the hyalinization of collagen, which produced injury of the valve bulk. The cases in which the necrotic tissue was replaced by young granulation tissue were charac-

died animal. The peak and trough concentrations of vancomycin were determined on day 3 by the fluorescence polarization immuno-

Results

In vitro susceptibility studies. The MICs and MBCs of the studied antibiotics for inocula of the MRSA strain of ~5 × 10^5 CFU/ml were 64 and 128 μg/ml, respectively, for oxacillin and 1 and 1 μg/ml, respectively, for vancomycin, while the MICs and MBCs for inocula of ~5 × 10^7 CFU/ml were 128 and 256 μg/ml, respectively, for oxacillin and 1 and 1 μg/ml, respectively for vancomycin. Time-kill studies done with 1 μg of vancomycin per ml demonstrated a reduction of the initial inoculum of ~5 × 10^6 CFU/ml to inocula at 6 and 24 h of incubation that were equivalent to 2.5 and 2.3 log_{10} CFU/ml, respectively. With an initial inoculum of ~5 × 10^7 CFU/ml, a reduction equivalent to 2.5 log_{10} CFU/ml was observed at 6 h of incubation, while after 24 h of incubation, an increase equiv-

Survival. Of the 70 rabbits used in the main study, three died within less than 24 h after placement of the catheter and seven were excluded from further analysis because they did not meet the inclusion criteria. Overall, 60 rabbits were evaluated for heart valve tissue sterilization and reduction of bacterial
counts. The mean survival rates of all study groups are presented in Table 1, while the Kaplan-Mayer survival curves are presented in Fig. 1. No statistically significant difference was observed in the survival rates between the group treated with vancomycin and the group treated with vancomycin plus dexamethasone. It is of interest that animals treated with dexamethasone alone exhibited a mean survival rate that was 2 days longer than that of the control animals. However, only three animals (21.5%) treated with dexamethasone were alive until the day of sacrifice.

**Bacteriological studies in animals. (i) Blood cultures.** For the pretreatment blood cultures, all study groups yielded comparable mean bacterial burdens (range, 2.61 ± 0.78 to 2.74 ± 1.04 log_{10} CFU/ml [mean ± SD]) (Table 1). During treatment with vancomycin or vancomycin plus dexamethasone, the few positive blood cultures found yielded mean bacterial burdens similar to those in the pretreatment cultures. In contrast, in the dexamethasone group, the mean bacterial burden on day 3 of treatment was statistically significantly higher than that of the pretreatment group (4.10 ± 0.96 log_{10} CFU/ml [n = 7] versus 2.71 ± 1.28 log_{10} CFU/ml [n = 7], respectively; P = 0.046). The numbers of sterile blood cultures on day 3 and day 5 of treatment in the group receiving vancomycin were statistically significantly higher than those of the pretreatment group (P = 0.002 and P < 0.001, respectively). The same was true for the group receiving vancomycin plus dexamethasone (P = 0.001 and P = 0.005, respectively). The number of sterile blood cultures on day 3 of treatment in the group receiving vancomycin was statistically significantly higher than that of the control group (P = 0.037) and that of the group receiving dexamethasone (P = 0.003). The respective P values for the results for the group receiving vancomycin plus dexamethasone were 0.061 (versus results for controls) and 0.009 (versus results for the dexamethasone group).

(ii) **Heart valve tissue cultures.** For the sterilization rate of heart valve tissue, the combination of vancomycin plus dexamethasone was more effective than no treatment (P = 0.002), while no statistically significant differences were found between no treatment and treatment with vancomycin (P = 0.088) (Table 1). It is of interest to mention the fact that for one untreated animal in the control group, bacterial colonies were present but not detected from a limited number of animals. Only statistically significant differences in numbers of sterile blood cultures were found by treatment group and by treatment regimen. It is of interest that animals treated with dexamethasone alone exhibited a mean survival rate that was 2 days longer than that of the control animals. However, only three animals (21.5%) treated with dexamethasone were alive until the day of sacrifice.

With respect to the reductions of the bacterial burdens measured in heart valve tissue (in mean log_{10} CFU/ml [mean ± SD]) (Table 1), the greater effectiveness of vancomycin treatment compared to that of either no treatment or treatment with dexamethasone was statistically significant (Table 1). There was no statistically significant difference in the reductions of the bacterial counts in heart valve tissue between the vancomycin group and the vancomycin-plus-dexamethasone group (P = 0.86).

In the two main groups of interest, namely, the group treated with vancomycin and the group treated with vancomycin plus dexamethasone, four animals (one in the first group...
and three in the second group) died before the scheduled day of sacrifice; because of this, we did a separate analysis excluding those four animals in order to eliminate any possible effects of the premature deaths in the results of the study. Again, no statistically significant differences were observed between the two groups regarding the sterilization rate of heart valve tissue ($P = 0.264$) and the mean bacterial burden (mean log$_{10}$ CFU per gram of heart valve tissue) ($P = 0.184$).

**Pathology results.** Histological examination of aortic valves revealed ulceration of endothelial surfaces and formation of thrombotic vegetations. The vegetations (Fig. 2) of all animals showed similar pictures, each consisting of acellular fibrin, platelets, and a matrix colonized by bacteria, as previously described (4, 5). Bacterial colonies of various densities were seen only in vegetations. Inflammatory infiltration was observed at the peripheries of the vegetations, particularly in the marginal area of each valve, and not in proximity to bacterial colonies (Fig. 2). The absolute numbers of PMNs in the different groups are given in Table 1. There were statistically significant differences observed in the results for the vancomycin and vancomycin-plus-dexamethasone groups ($P < 0.001$), with higher numbers of PMNs observed in the latter group. The same was true for comparisons between the control group and the dexamethasone group ($P < 0.001$). Finally, concerning the severity of valvular tissue damage (Fig. 3), it should be mentioned that a favorable effect was observed only in animals treated with vancomycin plus dexamethasone. Indeed, the majority of animals in the control group and in the dexamethasone- or vancomycin-treated groups had severe tissue damage. In contrast, the majority of animals treated with vancomycin plus dexamethasone had mild or moderate tissue damage, a finding that was also evident in the pilot study, where higher doses of dexamethasone were administered. A statistically significant difference was observed between the animals receiving only vancomycin and the animals receiving the combination of vancomycin plus dexamethasone in the severity of tissue damage of the valve, which was lesser in the latter group ($P < 0.001$). This favorable result was also evident after the four animals who had died before the scheduled day of sacrifice were excluded from analysis ($P = 0.001$). Statistically significant differences were also observed between the control group or the dexamethasone group and the vancomycin-plus-dexamethasone group; again, the differences favored the last group (Table 1).

**Vancomycin concentration in serum.** The mean peak concentration of vancomycin in serum was 72.7 µg/ml ($n = 8$), while the trough serum vancomycin concentrations were undetectable ($n = 8$). These serum drug concentrations differ from those usually observed in humans. The concentrations of vancomycin in serum from the vancomycin- and vancomycin-plus-dexamethasone-treated animals were similar.

**DISCUSSION**

From the results of the present study, it is obvious that with respect to the survival rate, the vegetation sterilization rate, and the mean bacterial burden in heart valve tissue, there are no differences between animals treated with vancomycin and those treated with vancomycin plus dexamethasone (0.5 mg/kg, twice a day). However, we found a beneficial effect in animals treated with dexamethasone plus vancomycin with regard to the tissue damage of the aortic valves. More specifically, tissue damage was more pronounced in the vancomycin-treated animals than in the animals treated with vancomycin plus dexamethasone, and this difference was statistically significant.

The mechanism responsible for this result is unclear. The most likely explanation is that the beneficial effect of dexamethasone is related to its effect on mediators of inflammation. Recent studies showed that downregulation of the responses of lymphocytes and macrophages by corticosteroids alleviates the outcome of sepsis caused by *S. aureus* (27). Sodequist et al. (25) showed that E-selectin, vascular cell adhesion molecule-1, and tumor necrosis factor alpha concentrations in serum increased in patients with staphylococcal endocarditis. Dexamethasone, due to its anti-inflammatory action, reduces tumor necrosis factor alpha levels (7, 36), and this action, by reducing the tissue damage, may be responsible for the protection of the endothelium of the aortic valve. In the experimental model used in the present study, it has been shown that dexamethasone increases peripheral blood granulocyte counts by approximately 50% during the first days of infection (2). Dexamethasone also increases granulocyte colony-stimulating-factor (G-CSF) levels (11), and this may be an additional mechanism that protects the aortic valve from extensive damage. However, G-CSF did not increase the clearance of methicillin-susceptible *S. aureus* from aortic valve vegetations in an experimental study (10), despite the fact that it stimulated leukocytosis in infected animals.

Leukocytes are the primary host defense against *S. aureus* infection (31). In the first study of neutrophil functions in patients with IE, Repine et al. (20) showed that the bactericidal activities of PMNs in untreated patients were significantly depressed but that they returned rapidly to normal during treatment. Bayer et al. (2, 3) showed that intravascular granulocyte influx plays a significant role in modulating the spontaneous clearance of bacteria in experimental tricuspid valve endocarditis due to *Pseudomonas aeruginosa*, while this trend toward spontaneous bacterial clearance is not observed in aortic valve vegetations, possibly due to the fact that the granulo-
cytes were distributed on the periphery of the vegetation and not in proximity to the bacterial colonies. However, studies by Meddens et al. (15) have suggested that PMNs play a modulating role by preventing *Streptococcus sanguis* bacteria from undergoing unbridled growth, even in aortic valve endocarditis. Dexamethasone failed to prevent the spontaneous intra-vegetation clearance of *P. aeruginosa* in a study by Bayer et al. (2), a finding in contradiction to those of a study of streptococcal tricuspid endocarditis (8). These disparate results possibly reflect differences in host defenses against gram-positive cocci and gram-negative bacilli (2). Other studies have confirmed the beneficial role of both monocytes and PMNs (16, 29) in the course of staphylococcal aortic valve endocarditis and septicemia (31). Moreover, Frank and Roth (9) have shown that the anti-inflammatory effects of corticosteroids may be partially mediated by factors released by monocytes. The findings of the above-mentioned studies are in accordance with the results of the present study, since the increased numbers of PMNs found in the animals treated with vancomycin plus dexamethasone compared with those found in animals treated with vancomycin only were associated with milder damage of the aortic valves. Nevertheless, PMNs alone are not able to reduce the intravegetation bacterial burden or the severity of tissue damage, since for the animals treated with dexamethasone alone, despite the high numbers of PMNs observed, the severity of tissue damage and the mean bacterial burden were comparable to those of the control animals. It is of interest that dexamethasone-treated animals, which showed a very high mean bacterial burden, exhibited a higher mean survival rate than did untreated controls. However, despite the fact that the mean survival rate of dexamethasone-treated animals was 2 days longer than the mean survival rate of the untreated animals, the vast majority of the former died before the day of sacrifice. This finding suggests that the beneficial effect of dexamethasone lasts for the first 4 days of treatment. In a study by Francioli and Freedman (8), mortality was not affected by dexamethasone in animals with streptococcal aortic valve endocarditis.

Regarding the findings of the histological examination, it is of interest that bacterial colonies were seen in the vast majority of vegetations (Table 1), even in those that did not yield bacteria in tissue culture. This is in disagreement with the findings of the study of Francioli and Freedman (8), where bacterial colonies of *S. sanguis* were seen when the vegetations contained more than 10^5 CFU/g. In the present study, inflammatory infiltration was more commonly seen in the dexametha-

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**FIG. 2.** Vegetation consists of acellular fibrin (thin arrow), platelets, and matrix colonized by bacteria (thick arrows). Inflammatory infiltrations are observed at the periphery of the vegetation (arrowheads), not in proximity to the bacterial colonies. Samples were stained with hematoxylin and eosin.
FIG. 3. The three degrees of valvular tissue (Va) damage in an area proximal to vegetation (Ve) are shown. (a) Mild damage, young granulation tissue (arrows). (b) Moderate damage, hyalinization in a narrow zone in proximity to the vegetation (arrows). (c) Severe damage, conversion of valvular tissue into densely hyaline (almost acellular) material (arrows). Samples were stained with hematoxylin and eosin.
sone-treated groups and was observed at the periphery of vegetations, particularly in the marginal area of each valve, and not in proximity to bacterial colonies; this finding is in accordance with those of the previously mentioned study (8).

In conclusion, the addition of dexamethasone to vancomycin for 5 days of treatment has a beneficial effect, i.e., a reduction in the severity of valve tissue destruction, in the treatment of experimental aortic valve endocarditis due to MRSA while it has no effect on either survival or blood or vegetation sterilization rates. However, the possibility that the steroids might delay valve destruction but not prevent it could not be excluded. In order to elucidate better this possibility, future studies—especially if data regarding heart function obtained by echocardiography (34) are available—with protocols that include treating animals to the point of cure and then observing them without treatment for some period before sacrifice are needed. The clinical relevance of the results of the present experimental study is the possibility that dexamethasone could reduce the severity of valve damage and possibly the need for surgical operation and valve replacement.

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REFERENCES


