Reduction of Bacterial Titers by Low-Dose Aspirin in Experimental Aortic Valve Endocarditis

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Using a rabbit model of *Staphylococcus aureus* endocarditis, we studied the effects of aspirin on the natural progression of this infection. Compared with untreated animals, the aspirin-treated animals showed a 30% (P = 0.11) reduction in the weight of the vegetations and an 84% (P = 0.03) reduction in the bacterial titer of the vegetations.

Since its introduction in the late 19th century, aspirin (acetylsalicylic acid [ASA]) has been used as a medicinal agent for a variety of ailments. Postoperative aspirin treatment has recently been shown to have a protective effect against prosthetic joint infection (20). This effect may be at least partly related to ASA inhibitory action on platelets, since many of the benefits associated with this therapy have been attributed to its ability to inhibit platelet aggregation by irreversibly acetylating cyclooxygenase and preventing the subsequent synthesis of thromboxane A_2 (13, 17).

Although platelets are recognized most often for their role in maintaining homeostasis, they may also function as an essential component in the eradication of infection (2, 9). In vitro, the interaction between platelets and bacteria results in platelet activation and irreversible aggregation. In vivo evidence from the rabbit endocarditis model suggests that the fibrin-platelet lesion on the damaged cardiac valve surface provides the nidus for the attachment of bacteria and the ensuing infection (3, 4, 6, 14). Since exposure of platelets to aspirin inhibits aggregation, it is reasonable to expect that aspirin will alter bacterial attachment to the endocardium. The purpose of this study was to evaluate the effect of ASA on the development of *Staphylococcus aureus* endocarditis.

(This study was presented at the American Society for Microbiology 92nd General Meeting, New Orleans, La., 26 May 1992 [12a].)

The methicillin-susceptible *S. aureus* (MSSA) strain used in this study was obtained from the blood of a patient with endocarditis. Female New Zealand White rabbits (Pines Acres Rabbitry, West Brattleboro, Vt.) weighing between 2 and 3 kg were used. Experimental endocarditis was induced with a polyethylene catheter by a modification of previously described techniques (1, 4, 6). The catheter was left in place throughout the experiment. When the catheter was removed at autopsy, only vegetations adherent to the aortic valve were considered for further study. Inclusion in the study required that proper placement of the catheter across the aortic valve be verified at autopsy.

Immediately after surgery, the animals were randomized to receive either ASA (5 mg/kg of body weight in 5 ml of water) or a control (5 ml of water) given via the oral-gastric

route. Either ASA or the control was administered every 24 h for a total of five doses. The pharmacokinetic profile of salicylic acid (SA) in the rabbit model was studied after the first (n = 6) and the fifth (n = 3) doses to verify that the drug was absorbed. Blood samples were drawn at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, and 6 h postdose. A high-performance liquid chromatography assay was used for the SA analyses (12). The linear range of SA in serum was 1 to 100 μg/ml, with a correlation coefficient of >0.998. The interday and intraday coefficients of variation for the check samples were ≤6.6%. Pharmacokinetic parameters were estimated by using a nonlinear least-squares fitting program (PCNONLIN; Statistical Consultants Inc., Lexington, Ky.).

At the time of the fifth dose (96 h after catheter insertion), each animal was inoculated via the marginal ear vein with a 1-ml suspension of MSSA containing 5×10^6 organisms. Final inoculum concentrations were confirmed by serial dilution and plating techniques.

Twenty-four hours after inoculation, the animals were sacrificed. The heart of each animal was removed by aseptic techniques, and the chambers of the left side were examined to confirm the presence of aortic vegetations. The vegetations for each rabbit were excised, pooled, washed once in sterile isotonic saline, blotted dry on sterile filter paper, and placed in preweighed microcentrifuge tubes. The tubes were then reweighed to obtain the weight of the vegetations. Bacterial titers (CFU counts) were determined by homogenizing the vegetations in 1 ml of sterile 0.9% sodium chloride. Serial dilution and plating techniques were used to determine the number of CFU present following incubation at 37°C for 24 h. The MICs of ASA and its major metabolite, SA (Sigma Chemicals, St. Louis, Mo.), for the MSSA (inoculum size of 5×10^5 CFU/ml) in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) were determined by a microdilution method (16).

Differences in vegetative weight and bacterial density were analyzed by a two-tailed, unpaired Student's t test. A P value of ≤0.05 was considered significant. All results are expressed as means ± standard deviations.

Forty-one animals underwent the catheterization procedure. Two animals in each group were excluded from the analysis because of a lack of vegetative growth despite adequate catheter placement, verified at the time of autopsy. The quantitative data with respect to vegetative weight and

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TABLE 1. Weights and bacterial densities of aortic valve vegetations

Treatment	No. of animals	Wt of vegetation (mg)[a,b]	Bacterial density ($\log_{10}$ CFU/g)[a,c]
Control	18	18.42 ± 9.76	9.14 ± 0.76
ASA	19	12.81 ± 10.68	8.28 ± 1.25

[a] Mean ± standard deviation.
[b] $P = 0.11$.
[c] $P = 0.03$. Three animals in each group showed sterile vegetations.

bacterial density for the study animals are displayed in Table 1. Data show a 30% reduction in the weight of the fibrin-platelet lesion ($P = 0.11$) and an 84% reduction in bacterial density ($P = 0.03$) in the ASA-treated group compared with those of the control animals. No difference was found in the concentration of the initial inoculum (mean $\log_{10}$ CFU per milliliter) between the control (6.45 ± 0.2) and ASA-treated (6.39 ± 0.3; $P = 0.54$) animals.

The pharmacokinetic parameter estimations for SA after the first and fifth doses are shown in Table 2. No statistically significant differences between the calculated parameters obtained at the beginning or at the end of the dosing regimen were noted. In addition, our dosing regimen produced a clinically relevant concentration-time profile (i.e., area under the concentration-time curve and maximum concentration of drug in serum) compared with the profile for the administration of a 325-mg ASA tablet in humans (11).

The MICs of ASA and SA for the standard inoculum of the MSSA strain exceeded the maximum concentration (500 µg/ml) tested, indicating no microbiologic effect of aspirin on the organism.

This study provides evidence that ASA caused a statistically significant reduction in the bacterial density (CFU per gram) of the vegetation compared with the density in control animals. This effect was evident despite the fact that both ASA and SA displayed no obvious antibacterial activity. This finding has also been observed in streptococci in a similar model of endocarditis (19). Aspirin has been shown to reduce the reactivity of platelets to a variety of stimuli, including arachidonic acid, collagen, platelet-activating factor, and bacteria (9, 18). Thus, it is suggested that ASA inhibits the bacterium-platelet interaction, thereby limiting the number of bacteria incorporated into the fibrin and platelet meshwork of the vegetation. However, because the aggregate patterns of platelets exposed to collagen and bacteria are quite similar and because previous study has shown that collagen-induced platelet activation is only partially inhibited by ASA, multiple mechanisms may be responsible for the observed effect (18). Therefore, the possi-

bility of ASA's effects on other important mediators of adherence, such as fibronectin and thrombospondin, cannot be overlooked (7, 8).

In this study, it was also observed that there was a reduction in weight of the valvular vegetation; however, it was not statistically significant. Pujadas et al. have shown previously that ASA at dosages of 1 and 10 mg/kg/day significantly reduced the weight of sterile thrombotic vegetations in the same model (15). The trend that our data showed is consistent with their findings. A dosage of 50 mg/kg/day resulted in a modest, although not statistically significant, reduction in weight, while 500 mg/kg/day had no effect on the weight of the vegetations (15). Other investigators report that ASA in a dosage of 50 mg/kg/day resulted in significant reductions, while higher dosages yielded no difference in the weight of the vegetations (10, 19). Since the weight of the vegetation is not influenced by high-dose ASA therapy, it appears that inhibiting platelet aggregation is not the sole mechanism of action of ASA. The efficacy of low-dose therapy may be related to its ability to prevent the generation of thromboxane without affecting vascular prostacyclin (5).

The reduction in vegetation weight may have important clinical implications for decreasing the incidence of embolic phenomena commonly experienced with endocarditis. Additionally, large vegetations require a longer duration of therapy for sterilization; therefore, the reduction in size may allow an abbreviated course of antibiotics. With the reduction in vegetative size and bacterial density, bacterial exposure to administered antibiotics could be enhanced, sterilization of the vegetation should be faster, and clinical outcome should improve.

TABLE 2. Pharmacokinetic parameters of SA after 5-mg/kg dose of ASA[a]

Dose (n)	$t_{1/2}$ (h)	AUC (mg · h/liter)	T_{max} (h)	C_{max} (mg/liter)
First (6)	4.97 ± 2.08	104.32 ± 41.87	0.98 ± 0.43	13.61 ± 6.91
Fifth (3)	5.73 ± 3.82	86.85 ± 39.80	1.7 ± 0.7	9.25 ± 2.23

[a] Means ± standard deviations. Differences in the calculated parameters for the first and fifth doses are not statistically significant (Student's t test). Abbreviations: $t_{1/2}$, half-life; AUC, area under concentration-time curve; T_{max}, time to maximum concentration of drug in serum; C_{max}, maximum concentration of drug in serum.

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