ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1995, p. 1748–1751 Vol. 39, No. 8
0066-4804/95/$04.00+0

Influence of Aspirin on Development and Treatment of Experimental *Staphylococcus aureus* Endocarditis

DAVID P. NICOLAU,[1,2,3]* MARKOS N. MARANGOS,[1,2] CHARLES H. NIGHTINGALE,[1,3] AND RICHARD QUINTILIANI[2,4]

Department of Pharmacy[1] and Division of Infectious Diseases and Allergy/Immunology,[2] Hartford Hospital, Hartford, Connecticut 06102; School of Pharmacy, University of Connecticut, Storrs, Connecticut 06268[3]; and School of Medicine, University of Connecticut, Farmington, Connecticut 06032[4]

Received 13 March 1995/Returned for modification 16 May 1995/Accepted 5 June 1995

Previously, we have shown that a 5-mg/kg of body weight daily dose of aspirin (ASA) caused reductions in the bacterial densities and weights of aortic vegetations in a rabbit model of *Staphylococcus aureus* endocarditis. We sought to determine (i) whether ASA dosage influences the development of vegetations and (ii) whether ASA given with antimicrobial therapy improves the treatment outcome of infective endocarditis. To study the influence of ASA dosage, animals received either no ASA (control) or oral doses of 2.5, 10, 20, and 50 mg/kg daily. The 2.5- and 10-mg/kg groups had statistically significant reductions in vegetation weight compared with untreated controls. The 10-mg/kg dose also resulted in a significant decrease in bacterial densities compared with those of the controls. Although reductions in weight and bacterial density were observed in other ASA-treated groups, these did not achieve statistical significance. To study the influence of ASA and antimicrobial therapy, the animals received either vancomycin alone or vancomycin with ASA. When ASA was given prior to and during antimicrobial therapy, a significant reduction in vegetation weight was observed. Additionally, the rate of sterilization was directly proportional to this observed reduction in weight. ASA's impact on the reduction of both the bacterial density and the weight of aortic vegetations is a dose-dependent phenomenon. When given with antimicrobial therapy, ASA not only reduces vegetation weight but also improves the rate of sterilization. This study provides additional data regarding the role of ASA in the treatment of endocarditis.

The pathogenesis of infective endocarditis is complex and requires the interaction of bacteria, platelets, numerous plasma factors, and integrin-binding proteins on the surface of the damaged endothelium. Once the consolidation of the vegetation components has become complete, the infecting organisms exist at high densities in fibrin and platelet meshwork (1, 2). This meshwork not only provides bacterial protection from the host defenses but also creates an environment in which the bacteria exist in a state of reduced metabolic activity and are, therefore, less susceptible to antimicrobial therapy. Because platelets are an integral component of the vegetation, platelet-induced dysfunction may offer a target site for limiting the induction and progression of this disease.

Aspirin (ASA) has long been recognized as an agent that produces platelet dysfunction when it is taken orally. Within platelets, ASA blocks the synthesis of thromboxane A_2, which is a vasoconstrictor and a promoter of platelet aggregation, by irreversibly inhibiting the cyclooxygenase and hydroperoxidase reactions needed for the production of thromboxane A_2 (15). Once induced, this defect cannot be repaired during the life span of the platelets because these cells lack the necessary biosynthetic machinery to produce new protein. As a result, the only way for the cyclooxygenase to recover is via the production of new platelets, which also explains why ASA can be fully effective as an antiplatelet agent with a once-daily administration. Therefore, the administration of relatively small daily doses is simply to affect the newly produced platelets since the last dose (9).

* Corresponding author. Mailing address: Division of Infectious Diseases, Hartford Hospital, 80 Seymour St., Hartford, CT 06102. Phone: (203) 545-3941. Fax: (203) 545-5112.

Previously, we have shown that a dose of 5 mg of ASA per kg of body weight per day in rabbits, which produced clinically achievable concentrations in humans, can affect the development of *Staphylococcus aureus* aortic valve endocarditis (8). The ASA-treated animals showed a 30% reduction in the weight and an 84% decrease in the bacterial density of the vegetation, compared with the untreated controls. This effect was observed even though the ASA has no microbiologic activity against the pathogen used in the model.

The purposes of this study were (i) to determine whether the influence of ASA on the development of experimental *S. aureus* endocarditis is a dose-dependent phenomenon and (ii) to determine whether ASA when combined with an antimicrobial agent improves the outcome of this infectious process.

(This work was presented previously [8a].)

MATERIALS AND METHODS

Test organism and in vitro susceptibilities. The isolate used in this study was a methicillin-susceptible strain of *S. aureus* (MSSA [ATCC 29213]). The MICs of ASA, its major metabolite salicylic acid (SA), vancomycin, and combinations of ASA or SA with vancomycin against the MSSA were determined by the microdilution method (11). All tests were performed with Mueller-Hinton broth supplemented with Ca and Mg (Difco Laboratories, Detroit, Mich.). The MIC was defined as the lowest concentration of drug at which the microorganism does not show visible growth following 24 h of incubation at 37°C.

Production of endocarditis. Conventional female New Zealand White rabbits (*Oryctolagus cuniculus*; Pines Acres Rabbitry, West Brattleboro, Vt.) were obtained and cared for according to the guidelines provided by U.S. Department of Health and Human Services (7). Animals were allowed food and water ad libitum; however, they were made to fast from 3 h prior to and until 3 h after each ASA dose. Experimental endocarditis was induced by modifications of previously described techniques (3, 4). Briefly, the animals were anesthetized by the administration of 1.25 ml of ketamine-acepromazine (10:1 dilution of Ketaset [100 mg/ml] and PromAce [10 mg/ml]; Aveco, Fort Dodge, Iowa) per kg. The right internal carotid artery was exposed and opened between ligatures. Sterile aortic vegetations were induced by loosening the distal ligature and inserting a poly-

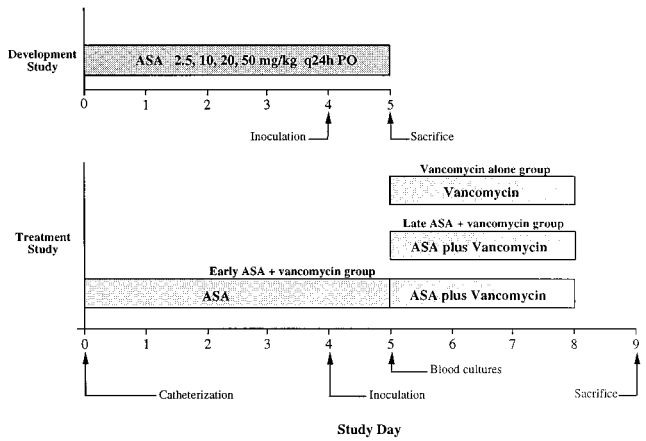

FIG. 1. Schematic of study interventions and drug therapy for both the ASA dosage-ranging study and combination with antimicrobial therapy. q24h, every 24 h; PO, postoperation.

ethylene catheter (external diameter, 1.09 mm; Becton Dickinson, Parsippany, N.J.) into the vessel and passing it toward the heart until pulsation and resistance indicated that it was across the aortic valve. 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. Infective endocarditis was produced by using a 1-ml suspension of MSSA containing 5×10^6 organisms inoculated via the marginal ear vein during the study of the influence of ASA dosage on vegetation development. In addition, several different bacterial challenges were studied for the treatment phase; however, as a result of unacceptable mortality (e.g., >60%) 24 h postinoculation, the final inoculum of MSSA was lowered by 2 log units to 5×10^4.

Influence of ASA dosage on the development of endocarditis. Once they had been catheterized, the animals were randomized to one of the following treatment groups: control (no ASA) or 2.5, 10, 20, or 50 mg of ASA (Sigma Chemicals, St. Louis, Mo.) per kg. ASA suspended in 5 ml of water was administered by the oral-gastric route via an orogastric tube once daily at 0900 h. These doses were selected to cover a dose range both greater than and less than the 5-mg/kg dose which had previously been shown to alter the development of aortic vegetations in this model (8). At 96 h after the placement of the catheter (fifth ASA dose), the animals were inoculated via their marginal ear veins with 5×10^6 MSSA cells (Fig. 1). Final inoculum concentrations were confirmed by serial dilution and plating techniques. Twenty-four hours later, the animals were sacrificed by an intravenous injection of pentobarbital and then by potassium chloride. At the time of sacrifice, the hearts were removed by aseptic techniques, and the chambers of the left side were examined to confirm the presence of aortic vegetations. The vegetations of each rabbit were excised, pooled, washed in sterile isotonic saline, and blotted dry on sterile filter paper. Vegetations were then homogenized 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 48 h. The vegetation was considered sterile (sensitivity limit, 100 CFU per vegetation) when the culture showed no growth after the incubation period. Culture-negative specimens were considered to contain 100 CFU for numerical purposes and for comparison with other treatment regimens.

Treatment of endocarditis. Endocarditis was produced as described above with a final inoculum of MSSA containing 5×10^4 organisms administered 96 h after the placement of the catheter. Figure 1 presents a schematic of study procedures and drug administration for this section of the investigation. The animals were randomized into three groups: vancomycin alone, late administration of ASA (late ASA) plus vancomycin, or early administration of ASA (early ASA) plus vancomycin. For all groups, vancomycin was administered intravenously at a dosage regimen of 50 mg/kg every 8 h, which was initiated 24 h after inoculation and continued for 72 h. In both ASA regimens, a dosage of 10 mg/kg was administered once daily via the orogastric route. This ASA dosage was selected on the basis of the first part of this study, which showed that 10 mg/kg/day provided optimal reduction of both vegetative weight and bacterial density. Early ASA was initiated immediately after catheterization, while late ASA was started simultaneously with vancomycin treatment. Prior to the initiation of antimicrobial therapy, blood cultures were obtained; animals with negative cultures after 48 h were discontinued from the study. Twelve hours after the last vancomycin dose, animals were sacrificed, and the vegetations were harvested, weighted, and homogenized for quantitative culture as described previously.

Statistical analysis. The weights and bacterial densities of the aortic valve vegetations from different treatment groups were compared by an analysis of variance method and then by the Scheffe test for multiple comparisons. The percentage of sterile vegetations in the treatment study was assessed by the chi-square test. A P value of ≤ 0.05 was considered significant. The results were expressed as means ± standard deviations (SD).

TABLE 1. Aortic vegetation weights and bacterial densities (means ± SD) after various doses of ASA

Dose (mg/kg)	Vegetation wt (mg)	Bacterial density ($\log_{10}$ CFU/g)
Control (no aspirin)	13.36 ± 4.93	8.77 ± 1.23
2.5	5.56 ± 2.89[a,b]	7.32 ± 1.56
10	7.36 ± 4.97[a]	7.21 ± 1.30[c]
20	11.22 ± 6.16	8.04 ± 1.72
50	9.76 ± 5.27	8.04 ± 1.46

[a] Significantly different from the control ($P = 0.0001$).
[b] Significantly different from the 20-mg/kg group ($P = 0.0001$).
[c] Significantly different from the control ($P = 0.0084$).

RESULTS

In vitro susceptibilities. The MICs of ASA and SA against the study isolate exceeded the maximum concentration (375 μg/ml) tested, thereby indicating that these agents have no appreciable microbiological activity against the organism. In addition, our previous pharmacokinetic analysis of the ASA dosages used in this dosage-ranging study revealed that the maximum concentrations in rabbits ranged from 7.54 to 70.00 μg/ml for the 2.5- to 50-mg/kg regimens (6). These pharmacokinetic data suggest that the resultant concentrations of ASA or SA in vivo are much lower than the upper limit tested in these in vitro experiments. The MIC of vancomycin against this isolate was 1 to 2 μg/ml, and the combination of ASA or SA with vancomycin revealed no alterations in the MIC of vancomycin compared with that of vancomycin alone. These data suggest that the addition of ASA or SA does not enhance the microbiological activity of this antimicrobial agent.

Influence of ASA dosage on the development of endocarditis. Ninety-three animals successfully completed this portion of the protocol (control [$n = 18$], and 2.5 [$n = 18$]-, 10 [$n = 20$]-, 20 [$n = 18$]-, and 50 [$n = 19$]-mg/kg ASA groups]. There were no differences in the concentrations of the initial inoculum between treatment groups. The relationships between vegetation weight (in milligrams) and bacterial density ($\log_{10}$ CFU per gram) versus the ASA dosage are shown in Table 1. The 2.5- and 10-mg/kg treatment groups had a statistically significant reduction in vegetative weight compared with the untreated controls ($P = 0.0001$). In addition, the 2.5-mg/kg dose also resulted in a statistically significant reduction in weight compared with the 20-mg/kg group ($P = 0.0001$). The 20- and the 50-mg/kg groups showed a reduction; however, this was not statistically significant.

Regarding the bacterial density, the 10-mg/kg dose resulted in a significant decrease compared with untreated controls ($P = 0.0084$). Although reductions were also evident in the other treatment groups compared with controls, these reductions did not reach statistical significance.

Treatment of endocarditis. Animals were excluded in cases of negative pretreatment blood cultures, perioperative death, or incorrect placement of the catheter across the aortic valve. Forty animals successfully completed this portion of the protocol (vancomycin alone [$n = 14$], late ASA plus vancomycin [$n = 13$], and early ASA plus vancomycin [$n = 13$]). No significant differences in the concentrations of the initial inoculum between treatment groups were noted. The aortic vegetation weights (means ± SD) for the vancomycin, late ASA plus vancomycin, and early ASA plus vancomycin groups were 11.69 ± 5.55, 8.65 ± 4.00, and 5.24 ± 3.08 mg, respectively.

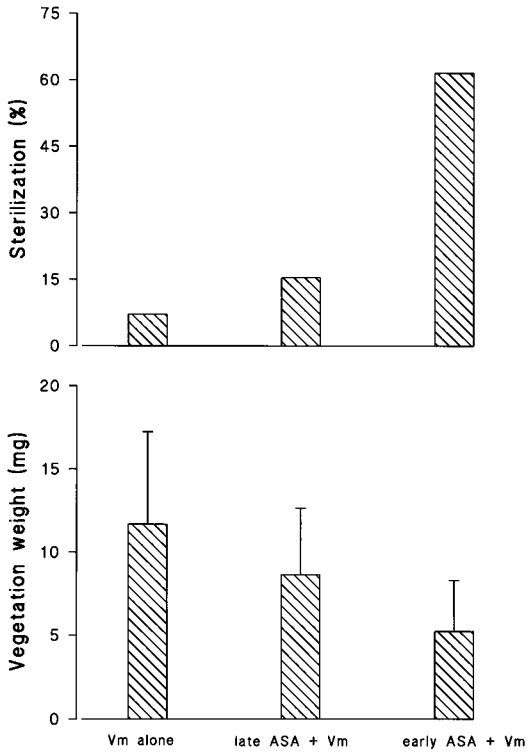

FIG. 2. Vegetation weights (means ± SD) and percentages of sterile aortic valve vegetations during treatment with vancomycin (Vm) alone or in combination with ASA.

Analysis of these data revealed a significant reduction in the weights of the early ASA plus vancomycin group compared with those treated with vancomycin alone ($P = 0.002$). Although a significant difference was not observed between the late ASA plus vancomycin group and animals randomized to vancomycin alone, we observed a mean reduction in vegetative weight that was proportional to the length of ASA treatment (Fig. 2).

The percentages of sterile aortic vegetations for the vancomycin, late ASA plus vancomycin, and early ASA plus vancomycin groups were 7.14, 15.38, and 61.54%, respectively. Eight days of ASA therapy (early ASA) combined with vancomycin significantly improved the sterilization rate compared with both vancomycin alone ($P = 0.01$) and late ASA plus vancomycin ($P = 0.04$). The percentage of sterile vegetations was directly proportional to the observed reduction in weight (Fig. 2).

DISCUSSION

The rabbit endocarditis model has been used to show that the combination of platelets and fibrin provide the nidus for the attachment of the bacteria and the ensuing infection. However, the role of the platelets in the induction and propagation of infective endocarditis is not yet well understood. Platelets appear to provide an adherence surface for the bacteria and may contribute to the overall vegetation mass. On the other hand, recent studies have shown that the platelets may antagonize the development of infective endocarditis through the secretion of several factors, such as the platelet microbicidal protein (PMP) that show bactericidal activity against pathogens commonly associated with endocarditis (12, 16).

ASA diminishes the aggregation response of platelets to several factors including bacteria, platelet-activating factor, and collagen. Our results show that ASA causes a dose-dependent reduction in the weights of the aortic vegetations. Doses of 2.5, 10, 20, and 50 mg/kg, which result in clinically achievable concentrations in humans (6), resulted in percentage reductions from the control value of 58, 45, 16 and 27%, respectively. The results of the present study are consistent with our previous observations and with the report described by Pujadas et al., who showed that ASA dosages of 1 and 10 mg/kg/day caused significant decreases in the weights of sterile thrombotic vegetations, whereas higher doses did not (8, 10). However, others have reported that an ASA dose of 50 mg/kg resulted in statistically significant reduction in vegetation weight by using a similar model of experimental endocarditis (5). The 10-mg/kg ASA treatment group also showed a significant reduction (97% compared with untreated controls) in bacterial density. This effect was observed despite the lack of apparent antibacterial activity of both ASA and SA.

The treatment study also reveals that when ASA is combined with vancomycin, it not only reduces the vegetation weight but also improves the sterilization rate of aortic valve vegetations infected with *S. aureus*. The increased sterilization rate may be explained because ASA reduces the bacterial density and size of the vegetation and therefore not only lowers the total microbial load within the vegetation but also increases the potential exposure of organism to the antimicrobial agent via improved penetration and distribution throughout the infection site.

Although the exact mechanism for these observations has not been fully elucidated, the dose-dependent effect suggests that the mechanism involves more than just the prevention of platelet aggregation, for this can be accomplished with a variety of doses (14). Although these observations may be due in part to ASA's direct effect on platelets, it is likely that this agent's effect on prostacyclin and mediators of adherence, such as thrombospondin and fibronection, also contributes to the observed reduction in both bacterial density and vegetation weight. In addition, the reduction in vegetation weight observed with ASA therapy may have other important clinical implications, such as decreasing the incidence of embolic phenomena that are commonly experienced with endocarditis (13). Although many questions remain and other investigations are required to determine the mechanism responsible for our observations, this study provides encouraging data regarding the role of ASA therapy in the treatment of infective endocarditis.

ACKNOWLEDGMENTS

We thank the members of the animal care facility at our institution for their care of the animals through the study period.

This study was supported in part by a grant from Hartford Hospital (HH-1017-80-050).

REFERENCES

1. **Clawson, C. C., and J. G. White.** 1971. Platelet interaction with bacteria. I. Reaction phases and effects of inhibitors. Am. J. Pathol. **65:**367–380.
2. **Durack, D. T., and P. B. Beeson.** 1972. Experimental bacterial endocarditis. I. Colonization of a sterile vegetation. Br. J. Exp. Pathol. **53:**44–49.
3. **Durack, D. T., P. B. Beeson, and R. G. Petersdorf.** 1973. Experimental bacterial endocarditis. III. Production and progress of the disease in rabbits. Br. J. Exp. Pathol. **54:**142–151.
4. **Garrison, P. K., and L. R. Freedman.** 1970. Experimental endocarditis. I. Staphylococcal endocarditis in rabbits resulting from placement of a polyethylene catheter in the right side of the heart. Yale J. Biol. Med. **42:**394–410.
5. **Levison, M. E., J. Carrizosa, D. Tanphaichitra, P. K. Schick, and W. Rubin.** 1977. Effect of aspirin on the thrombogenesis and on production of experimental aortic valvular *Streptococcus viridans* endocarditis in rabbits. Blood **49:**645–650.

6. **Marangos, M. N., C. O. Onyeji, D. P. Nicolau, and C. H. Nightingale.** 1995. Disposition of aspirin in female New Zealand White rabbits. Lab. Anim. Sci. **45:**67–69.
7. **National Institutes of Health.** 1985. Guide for the care and use of laboratory animals. NIH publication no. 85-23. U.S. Department of Health and Human Services, Washington, D.C.
8. **Nicolau, D. P., C. D. Freeman, C. H. Nightingale, R. Quintiliani, C. J. Coe, E. G. Maderazo, and B. W. Cooper.** 1993. Reduction of bacterial titers by low-dose aspirin in experimental aortic valve endocarditis. Infect. Immun. **61:**1593–1595.

8a. **Nicolau, D. P., M. N. Marangos, C. H. Nightingale, and R. Quintiliani.** 1994. Program Abstr. 94th Gen. Meet. Am. Soc. Microbiol. 1994, A 6 and B 239. American Society for Microbiology, Washington, D.C.

9. **Patrono, C.** 1994. Aspirin as an antiplatelet drug. N. Engl. J. Med. **330:**1287–1293.
10. **Pujadas, R. E., E. Escriva, F. Fernandez, J. Jane, J. Argimon, P. Fava, and M. C. Calera.** 1988. Effecto de diversas dosis de aspirina sobre el desarrolo de la endocarditis trombotica aseptica aortica inducia experimentalmente en el conejo. Rev. Esp. Cardiol. **41:**31–34.
11. **Sahm, D. F., and J. A. Washington II.** 1991. Antibacterial susceptibility tests: dilution methods, p. 1105–1116. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
12. **Sullam, P. M., F. Uwe, M. R. Yeaman, M. G. Tauber, A. S. Bayer, and H. F. Chambers.** 1993. Effect of thrombocytopenia on the early course of streptococcal endocarditis. J. Infect. Dis. **168:**910–914.
13. **Taha, T. H., S. S. Durrant, P. K. Mazeika, P. Nihoyannopoulos, and C. M. Oakley.** 1992. Aspirin to prevent growth of vegetations and cerebral emboli in infective endocarditis. J. Intern. Med. **231:**534–546.
14. **The Dutch TIA Trial Study Group.** 1991. A comparison of two doses of aspirin (30 mg vs. 283 mg a day) in patients after a transient ischemic attack or minor ischemic stroke. N. Engl. J. Med. **325:**1261–1266.
15. **Willard, J. E., R. A. Lange, and L. D. Hillis.** 1992. The use of aspirin in ischemic heart disease. N. Engl. J. Med. **327:**175–180.
16. **Wu, T., M. R. Yeaman, and A. S. Bayer.** 1994. In vitro resistance to platelet microbicidal protein correlates with endocarditis source among bacteremic staphylococcal and streptococcal isolates. Antimicrob. Agents Chemother. **38:**729–732.