Journal of Infection 90 (2025) 106389

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Letter to the Editor

Shikimic acid enhances vancomycin efficacy in experimental MRSA-induced infective endocarditis

Linezolid, vancomycin and daptomycin are considered the last-line of defense against infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). We recently read with great interest the study by Somawardana and colleagues,[1] which demonstrated that the combination of linezolid and alternating magnetic fields (AMF) significantly reduced MRSA biofilm formation and inflammation in a large animal model. Here, we describe that shikimic acid enhances the efficacy of vancomycin in a rat model of MRSA infective endocarditis. This combination therapy resulted in a marked reduction in inflammatory exudates and a significant decrease in MRSA densities and vegetation formation on the aortic valves.

Infective endocarditis (IE) is a life-threatening infection frequently caused by MRSA, especially in patients with prosthetic cardiac valve disease.[2] Despite the use of "last-resort" anti-MRSA antibiotics such as vancomycin or daptomycin, the mortality rate for IE remains alarmingly high. Notably, vancomycin monotherapy has shown limited effectiveness in treating severe MRSA endocarditis.[3] Therefore, there is an urgent need to explore alternative therapeutic options against such severe infection.

IE is characterized by vegetations composed of platelets, fibrin, and bacteria on the endocardial surface. Dual antiplatelet and antithrombin therapy has been shown *in vivo* to reduce endocarditis by inhibiting platelet binding. More recently, a single antiplatelet dose of ticagrelor was found to prevent *S. aureus*-infected vegetation formation in a mouse IE model.[4] Shikimic acid (SA) is a key precursor in the synthesis of the antiviral drug oseltamivir (Tamiflu), capable of reducing platelet activation and thrombus formation.[5] Given its antithrombotic and fibrinolytic activities, in this study, we evaluated (i) the MICs and *in vitro* activities of vancomycin alone or in combination with shikimic acid against the MRSA strain ATCC 43300; (ii) the *in vivo* efficacy of vancomycin and shikimic acid combination therapy compared to monotherapy in a prototypical experimental rat model of IE caused by MRSA; and (iii) the impact of combination therapy on inflammatory exudates and bacterial load in cardiac vegetations, as assessed by hematoxylin-eosin (HE) and Gram stainings.

The MICs of vancomycin and shikimic acid against MRSA ATCC 43300 were determined to be 1 mg/L and 3125 mg/L, respectively. Time-kill assays were performed using an initial inoculum of ~10^5 cfu/mL log-phase MRSA cells in the presence of vancomycin (0.5–1.5 mg/L) alone or in combination with shikimic acid (1560 mg/L). A concentration-dependent effect was observed when shikimic acid was combined with vancomycin at 0.5–1.5 mg/L (Fig. 1). Sub-MIC concentrations of shikimic acid or vancomycin alone were inactive and exhibited a growth pattern similar to the control (Fig. 1A). Vancomycin at 1.5 mg/L resulted in a transient growth delay for 9 h but was followed by a > 1.3 $\log_{10}$ cfu/mL regrowth at 24 h. However, when vancomycin concentrations of 1–1.5 mg/L of were combined with shikimic acid, a bacteriostatic or bactericidal effect was observed at 24 h, with a > 2.1-$\log_{10}$ cfu/mL reduction compared to either drug alone (Fig. 1B-C). Similar synergistic effects were noted when shikimic acid was combined with other antibiotics against MRSA.[6]

A well-characterized rat model of aortic IE was used for *in vivo* studies (Fig. 2A).[7] Female Sprague-Dawley rats (250 to 300 g) were obtained from Vital River Laboratory (Beijing, China). The Animal Research Committee (IACUC) of South China Agricultural University approved these studies (#2023C066). Rats underwent indwelling transcarotid-transaortic valve catheterization using polyethylene tubing (PE10, Becton Dickinson, Sparks, MD) and were infected intravenously with a 0.5 mL MRSA suspension delivering ~$10^{5.5}$ cfu/rat. At 24 h post-infection, rats were randomized to receive: (i) no therapy (control); (ii) vancomycin at 15 mg/kg intravenously (i.v.) twice a day

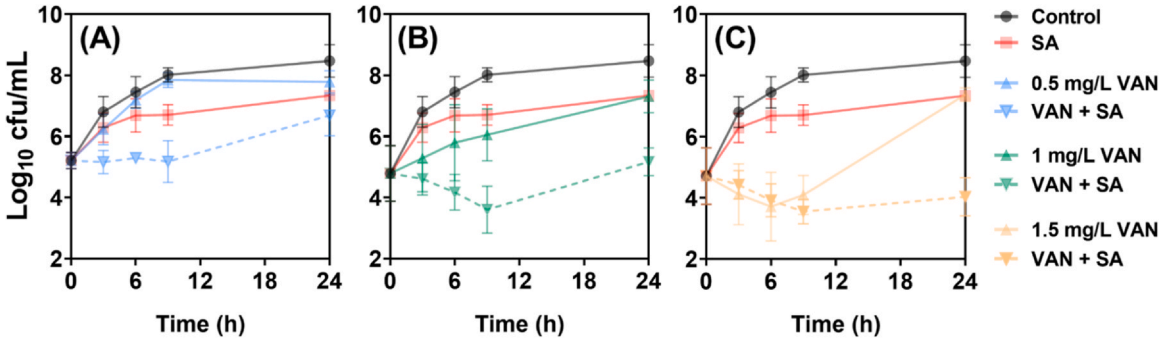
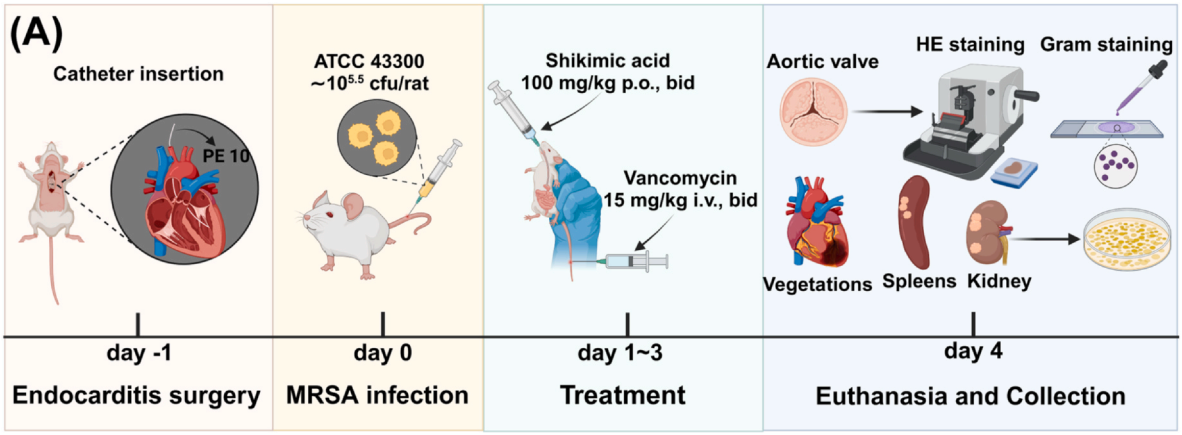
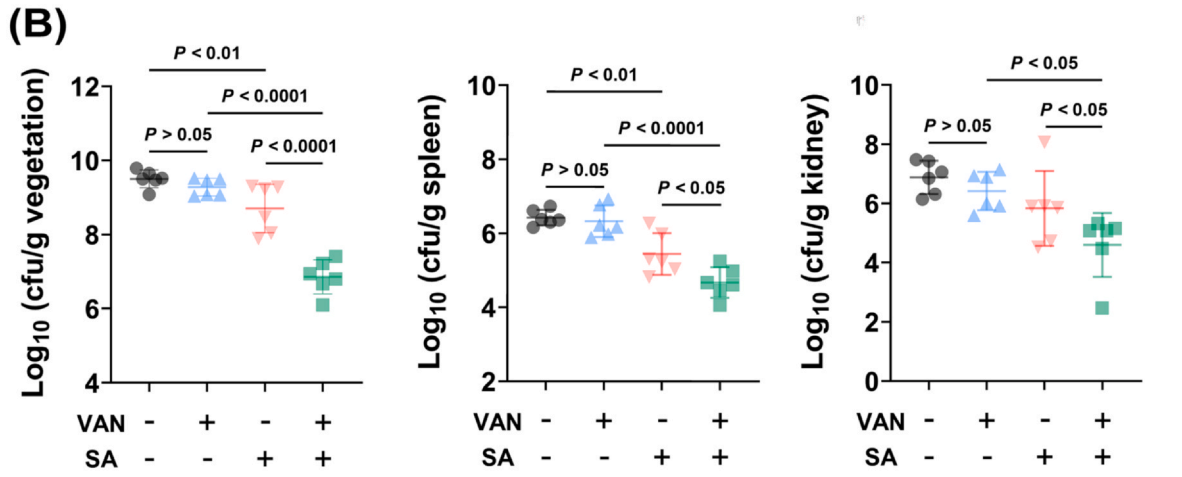


Fig. 1. *In vitro* effects of shikimic acid (SA; 1560 mg/L) alone or in combination with vancomycin (VAN) at concentrations of 0.5–1.5 mg/L against MRSA ATCC 43300. Data are expressed as the mean ± standard deviation (SD; n = 3–5).

https://doi.org/10.1016/j.jinf.2024.106389





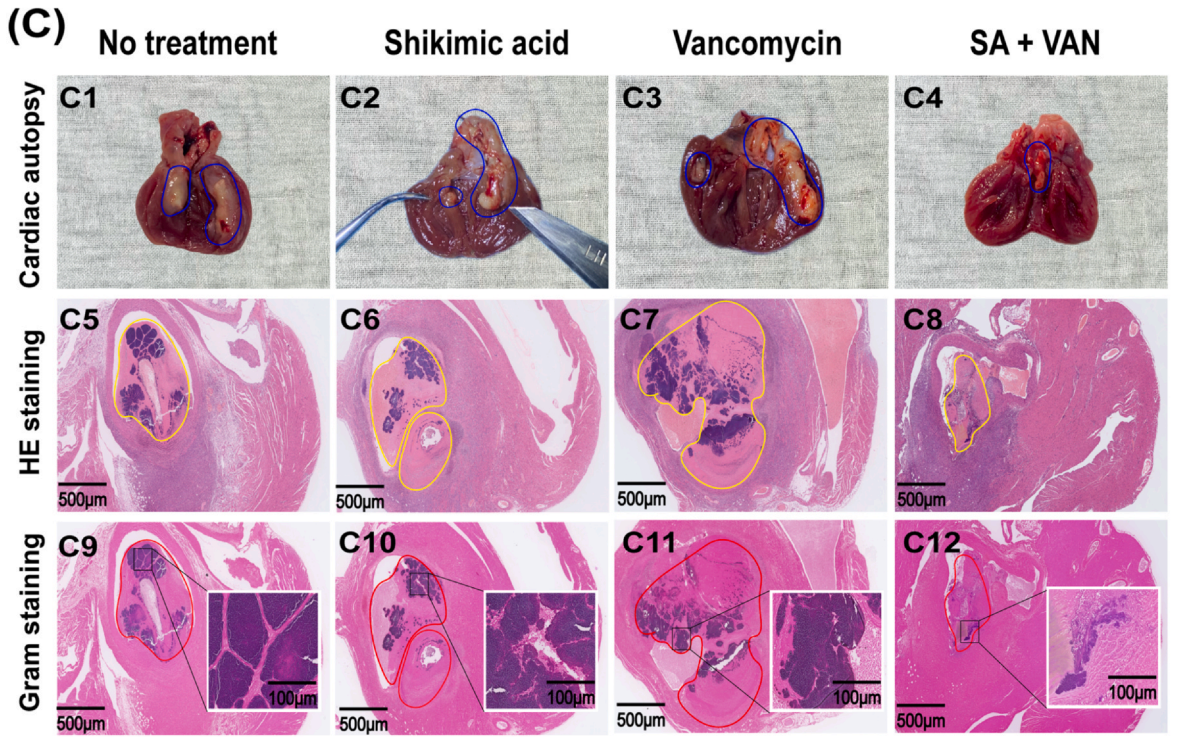


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Fig. 2. **(A)** Experimental protocols for the rat model of aortic infectious endocarditis (IE) due to MRSA. (**B**) MRSA densities in cardiac vegetations, spleens and kidneys of the IE model after 3 days of mono- and combination therapies with vancomycin (VAN; 15 mg/kg, i.v., bid) and shikimic acid (SA; 100 mg/kg, p.o., bid). Each dot represents an individual rat, and horizontal lines indicate the group mean (n = 6). Statistical comparisons were performed using an unpaired Student's *t*-test, with $P \leq 0.05$ considered statistically significant. **(C)** Comparative histopathological analysis of cardiac aortic valve tissue in the rat IE model after 3 days of mono- and combination therapies with vancomycin and shikimic acid. C1-C4, Autopsy images showing cardiac vegetations on the aortic valves (circled in blue). C5-C8, HE-stained sections of rat aortic valve tissue from each treatment group (2.5 × magnification), with yellow circles highlighting abnormal changes such as inflammatory exudates and anuclear, eosin-positive regions. C9-C12, Gram-stained sections of aortic valves (2.5 × magnification), with enlarged views (20×), highlighting clusters of Gram-positive bacteria (circled in red).

(bid); (iii) shikimic acid at 100 mg/kg orally (p.o.) bid; or (iv) a combination of shikimic acid and vancomycin. After 3 days of treatment, the rats were euthanized, and the target tissues (cardiac vegetations, kidneys and spleens) were aseptically removed and quantitatively cultured to assess bacterial loads, expressed as mean $\log_{10}$ cfu/g (± SD; n=6). Additional cardiac vegetation samples were isolated and subjected to HE and Gram stainings for histopathological analysis.

Consistent with previous findings,[8] animals with MRSA-induced IE showed no significant response to vancomycin monotherapy ($P > 0.05$; Fig. 2B). However, treatment with shikimic acid at 100 mg/kg resulted in statistically relevant ($P < 0.01$), but relatively modest reductions in MRSA densities in cardiac vegetations (> 0.8 $\log_{10}$ cfu/g) and spleens (> 1.0 $\log_{10}$ cfu/g) compared to untreated controls. Importantly, the combination of vancomycin and shikimic acid demonstrated significantly enhanced efficacy, with 1.7 to 2.6- $\log_{10}$ cfu/g reductions in MRSA counts in both cardiac vegetations and spleens ($P < 0.0001$; Fig. 2B) as compared with each monotherapy.

Macroscopic vegetations were observed on the aortic valves of untreated animals and all monotherapy groups (Fig. 2C1-C3). Pathological analysis of the cardiac valve showed that MRSA infection induced typical symptoms of IE, including severe infiltration of inflammatory cells into the valvular vegetations, valvar abscesses, platelet aggregation and thrombus formation on the heart valve surfaces (Fig. 2C5-C7; eosin-positive areas circled in yellow). Notably, combination therapy with shikimic acid and vancomycin significantly mitigated these IE symptoms, resulting in milder valvular lesions, reduced vegetation formation, and decreased infiltration of inflammatory cells (Fig. 2C4 and C8). These findings were further corroborated by Gram staining analysis, which showed a significantly lower bacterial density (Gram-positive spots circled in red) in the valvular vegetations of the combination therapy group compared to the monotherapy groups (Fig. 2C9-C12).

Current evidence indicates that all *S. aureus* strains capable of inducing bacteremia have the potential to cause IE.[9] While antibiotic therapy remains a mainstay of valve IE treatment,[10] increasing reports of vancomycin failures, even against susceptible *S. aureus* strains, highlight the urgent need for alternative therapies.[8] Although the precise mechanism is still unclear, our *in vivo* results suggest that shikimic acid, when used adjunctively with vancomycin, offers a promising experimental strategy for treating MRSA-induced IE in a rat model. Further studies are warranted to explore its clinical applicability.

In conclusion, the combination of shikimic acid and vancomycin demonstrates superior efficacy compared to vancomycin monotherapy for treating MRSA-induced IE, even with short-course therapy. Further studies are warranted to assess the safety and clinical applicability of this combination therapy.

Financial support

This work was jointly supported by grants from National Key Research and Development Program of China (2022YFD1802104), Guangdong Basic and Applied Basic Research Foundation (2024A1515010365), National Natural Science Foundation of China (32172909), Guangzhou Science and Technology Plan Project (2024A04J3412), Foundation for Innovative Research Groups of National Natural Science Foundation of China (32121004), Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2019BT02N054) and the 111 Center (D20008).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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