Antibacterial Activity of Ticagrelor in Conventional Antiplatelet Dosages Against Antibiotic-Resistant Gram-Positive Bacteria

Ticagrelor reversibly inhibits the platelet adenosine diphosphate P2Y12 receptor (P2Y12). It is approved for prevention of cardiovascular events in patients with atherosclerotic cardiovascular disease and shows evidence of superior clinical performance compared with other P2Y12 inhibitors. A post hoc analysis of the Comparison of Ticagrelor (AZD6140) and Clopidogrel in Patients With Acute Coronary Syndrome (PLATO) trial revealed that patients treated with ticagrelor had a lower risk of infection-related death than those treated with clopidogrel bisulfate. More recently, in the Targeting Platelet-Leukocyte Aggregates in Pneumonia With Ticagrelor (XANTHIPPE) study, ticagrelor was associated with improved lung function in patients hospitalized for pneumonia. We therefore questioned whether ticagrelor or its major metabolites could possess antimicrobial properties.

Methods | Ticagrelor and its major metabolites (M5 AR-C133913, M7, M8 AR-C124910) were synthetized and tested in time-kill assays against gram-positive methicillin-resistant Staphylococcus epidermidis RP62A (MRSE) (ATCC 35984); methicillin-sensitive Staphylococcus aureus (MSSA) (ATCC 25904, ATCC 6538); glycopeptide intermediate S aureus (GISA) Mu-50 (ATCC 700699); methicillin-resistant S aureus (MRSA) (ATCC BAA-1556); Enterococcus faecalis (ATCC 29212); vancomycin-resistant E faecalis (VRE) (ATCC BAA-2365); and Streptococcus agalactiae (ATCC 12386) and against gram-negative Escherichia coli (ATCC 8739) and Pseudomonas aeruginosa (PAK laboratory strain). Biofilm formation was assessed in vitro with crystal violet staining and in a mouse model of S aureus polyurethane-implant infection using Xen-29 bacteria (Perkin Elmer). Infected disks were implanted in specific pathogen-free BALB/cAnCrl mice (Charles River). The mouse protocol was approved by the ethical committee of Liège University.

Results | Ticagrelor and AR-C124910 had bactericidal activity against all gram-positive strains tested, including drug-resistant strains GISA, MRSE, MRSA, and VRE. The minimal bactericidal concentration was 20 μg/mL against MSSA, GISA, MRSA, and VRE; 30 μg/mL against MRSE; and 40 μg/mL against E faecalis and S agalactiae. Although a dosage of 5 μg/mL delayed growth of MRSA, ticagrelor was ineffective against gram-negative strains in concentrations up to 80 μg/mL. At minimal bactericidal concentration, ticagrelor was superior to vancomycin (Figure 1A), with rapid killing of late-exponential-phase cultures of MRSA (time to kill 99.9% of the initial inoculum, 2 hours). Bactericidal activity was similar to the bactericidal cyclic lipopeptide daptomycin, recently introduced against resistant strains of S aureus (Figure 1A). A subminimal bactericidal concentration of ticagrelor (10 μg/mL) combined with vancomycin (4 μg/mL) killed approximately 50% of the initial MRSA inoculum, depicting synergistic activity. Ticagrelor also increased the bactericidal activity of rifampicin, ciprofloxacin, and vancomycin in a disk diffusion assay. It displayed bactericidal activity against MRSE and VRE (Figure 1B and C), with superiority over vancomycin for killing MRSE. At 24 hours, its ability to kill MRSE and VRE was similar to daptomycin (Figure 1B and C). Ticagrelor inhibited MRSA, MRSE, and VRE biofilm formation in a dose-dependent manner (Figure 1D-F); biofilm mass was reduced by more than 85% after exposure to 20 μg/mL ticagrelor. Finally, in mice, conventional oral antiplatelet dosages of ticagrelor (3 mg/kg loading dose, then 1.5 mg/kg twice daily) inhibited biofilm growth on S aureus–preinfected implants and dissemination of bacteria to surrounding tissues (Figure 2).

Discussion | We describe bactericidal activity of ticagrelor against antibiotic-resistant gram-positive bacteria that pose a threat to human health. Although bactericidal concentrations are not reached systemically in patients receiving typical dosages for treating cardiovascular disease (ticagrelor Cmax = 1.2 μg/mL after one 180-mg loading dose and 0.75 μg/mL at 90 mg twice daily steady state), antibacterial activity at infection sites may still be achieved through local, possibly platelet-driven, drug accumulation. Our findings provide a mechanistic explanation for the reduced infection-related death with ticagrelor seen in the PLATO trial and could also explain improvement in lung function in patients with pneumonia who took ticagrelor in the XANTHIPPE study. These findings warrant further investigations, including design of randomized clinical trials comparing the protective activity of ticagrelor against gram-positive bacterial infection in patients with cardiovascular disease with other antiplatelet drugs. We are unaware of similar findings with other P2Y12 inhibitors, and we did not observe in vitro antibacterial activity of the active metabolite of prasugrel in concentrations up to 100 μg/mL. Ticagrelor might prove superior to other P2Y12 inhibitors in patients with cardiovascular disease at risk for gram-positive bacterial infections such as infective endocarditis. We did not isolate mutants resistant to ticagrelor, and serial passaging of MSSA or MRSA in the presence of subinhibitory concentrations of ticagrelor did not select for resistant mutants compared with ofloxacin or rifampicin, which is reassuring for long-term antiplatelet indications. There is a main limitation
Figure 1. In Vitro Characterization of Bactericidal and Anti-Biofilm Activity of Ticagrelor on MRSA, MRSE, and VRE

A-C, Time-kill curves with ticagrelor (20 μg/mL for MRSA and VRE; 45 μg/mL for MRSE), vancomycin (10 μg/mL), daptomycin (20 μg/mL for MRSA and VRE; 10 μg/mL for MRSE) or vehicle (1% DMSO). D-F, Mean biofilm biomass. Error bars indicate SD. CFU indicates colony-forming units; DMSO, dimethylsulfoxide; MRSA, methicillin-resistant Staphylococcus aureus; MRSE, methicillin-resistant Staphylococcus epidermidis; and VRE, vancomycin-resistant Enterococcus faecalis.

\[ a \] \( P < .05. \)

\[ b \] \( P < .01. \)

\[ c \] \( P < .001. \)

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in this study that will be addressed in future research. The in vivo demonstration of antibacterial activity of ticagrelor antiplatelet dosages was obtained in the mouse, which differs from humans in terms of ticagrelor pharmacokinetics. Notwithstanding, our findings also encourage future investigation of potential new ticagrelor-derived antibiotics, devoid of antiplatelet activity, against multiresistant staphylococci or enterococci.

Figure 2. In Vivo Bactericidal Activity of Ticagrelor in a Mouse Subcutaneous Foreign-Body *Staphylococcus aureus* Infection Model

A, Representative images of implanted mice before treatment and 24 hours after treatment with ticagrelor or vehicle. B, Mean values of bioluminescent signals. Error bars indicate SD. C, Skin sections (Gram stain [Sigma-Aldrich], original magnification ×6.5). Blue areas represent bacteria. Bars = 50 μm. p/s indicates photon per signal.
COMMENT & RESPONSE

We Can Learn From the Past, but We Must Pave the Future of Congenital Heart Disease Research

To the Editor—We read with interest the report by Smith et al1 titled “Long-term Outcomes of Tetralogy of Fallot: A Study From the Pediatric Cardiac Care Consortium.” In a retrospective multicenter cohort of 3283 participants, the authors aimed to determine the transplant-free survival of patients with tetralogy of Fallot (TOF) and the predictors of survival. The study showed excellent long-term survival following TOF surgical repair (94.5% at 25 years). Surgical era, operative strategy, and presence of genetic abnormality were independent predictors of survival. We want to congratulate the authors on completing this study that informs the management of patients with TOF where evidence is lacking. Low prevalence, broad spectrum of presentation, and heterogeneity in management make clinical research on congenital heart disease challenging. A retrospective study design and registry data overcome these challenges; however, they impose limitations that warrant highlighting.

First, the ability to assess all important outcomes are limited in retrospective studies. In this case, the authors focused on transplant-free survival as the primary outcome. Other outcomes important to patients should be evaluated, such as cardiac reintervention, cardiovascular hospitalizations, heart failure, exercise intolerance, and quality of life.

Second, it is often not possible to adjust for all important confounders using registry data, where information on important baseline characteristics, such as preoperative cardiac anatomy, is not available. According to international registries, about 60% of TOF repairs follow the traditional ventriculotomy and transannular patch strategy.2,3 We conducted a survey of surgeons that revealed that the choice of surgical strategy was strongly driven by personal and institutional preferences rather than contemporary evidence.4 Thus, we wonder whether the 3-fold increase in risk of death with the non-valve sparing approach is because of higher-risk TOF phenotype rather than to the repair strategy itself. Again, specifics of phenotype in these complex cases may be poorly captured in registries.

These challenges can be overcome in an international prospective cohort of patients with TOF enrolled prior to surgical interventions. In the Tetralogy of Fallot for Life cohort (NCT02968264), we collect baseline echocardiographic data to capture the TOF severity preoperatively.5 Operative details, such as the size of a transannular patch, are captured to describe the variability in repair strategy. Clinical and imaging follow-up for 2 years will provide information on right ventricular remodeling and clinical outcomes after various repair strategies. In addition, the results of this prospective cohort will be generalizable to the global TOF population, including geographical areas where TOF is more prevalent than North America.

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