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Ticagrelor, but not clopidogrel active metabolite, displays antithrombotic properties in the left atrial endocardium

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Aims Oral anticoagulation is considered standard therapy for stroke prevention in atrial fibrillation (AF). Endocardial activation triggers expression of pro-thrombotic mediators including tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1), and contributes to thrombus formation in the left atrial appendage (LAA) of AF patients. Recently, pleiotropic effects of specific P2Y12 receptor antagonists were demonstrated; however, whether these drugs possess antithrombotic effects on LAA endocardial cells currently remains unknown.

Methods and results LAA were obtained from 14 patients with known AF undergoing elective cardiac surgery including LAA removal at the University Hospital Zurich. LAA endocardial cells were isolated and pre-incubated with ticagrelor (10^{-7}, 10^{-6}, 10^{-5}M) or clopidogrel active metabolite (CAM) (1.5×10^{-8}, 1.5×10^{-7}, 1.5×10^{-6} M) before stimulation with tumour necrosis factor-alpha (TNF-α) (10 ng/mL). Finally, TF and PAI-1 expression and activity were analysed. Ticagrelor, unlike CAM, concentration dependently decreased TNF-α-induced TF expression and TF activity in LAA endocardial cells. Further, ticagrelor, but not CAM reduced PAI-1 expression and enzyme activity in TNF-α-stimulated LAA endocardial cells. In contrast, TF pathway inhibitor (TFPI) remained unaffected by both dugs.

Conclusion Ticagrelor, but not CAM, reduces expression and activity of TF and PAI-1 in LAA endocardial cells isolated from patients with AF, indicating possible local antithrombotic effects. Such pleiotropic properties of ticagrelor may contribute to a reduction in thromboembolic complications in patients with AF.

Keywords Atrial fibrillation • Clopidogrel • Endothelial activation • Thrombosis • Ticagrelor

Introduction

Atrial fibrillation (AF) is a common cardiac arrhythmia affecting approximately 2% of the general population in developed countries.[1] It increases the risk of thromboembolic complications such as cardiogenic stroke as well as heart failure and all-cause mortality.[2] Oral anticoagulation is superior to dual or single antiplatelet therapy[3] and is considered standard therapy to reduce thromboembolic complications and mortality in AF patients.[2]

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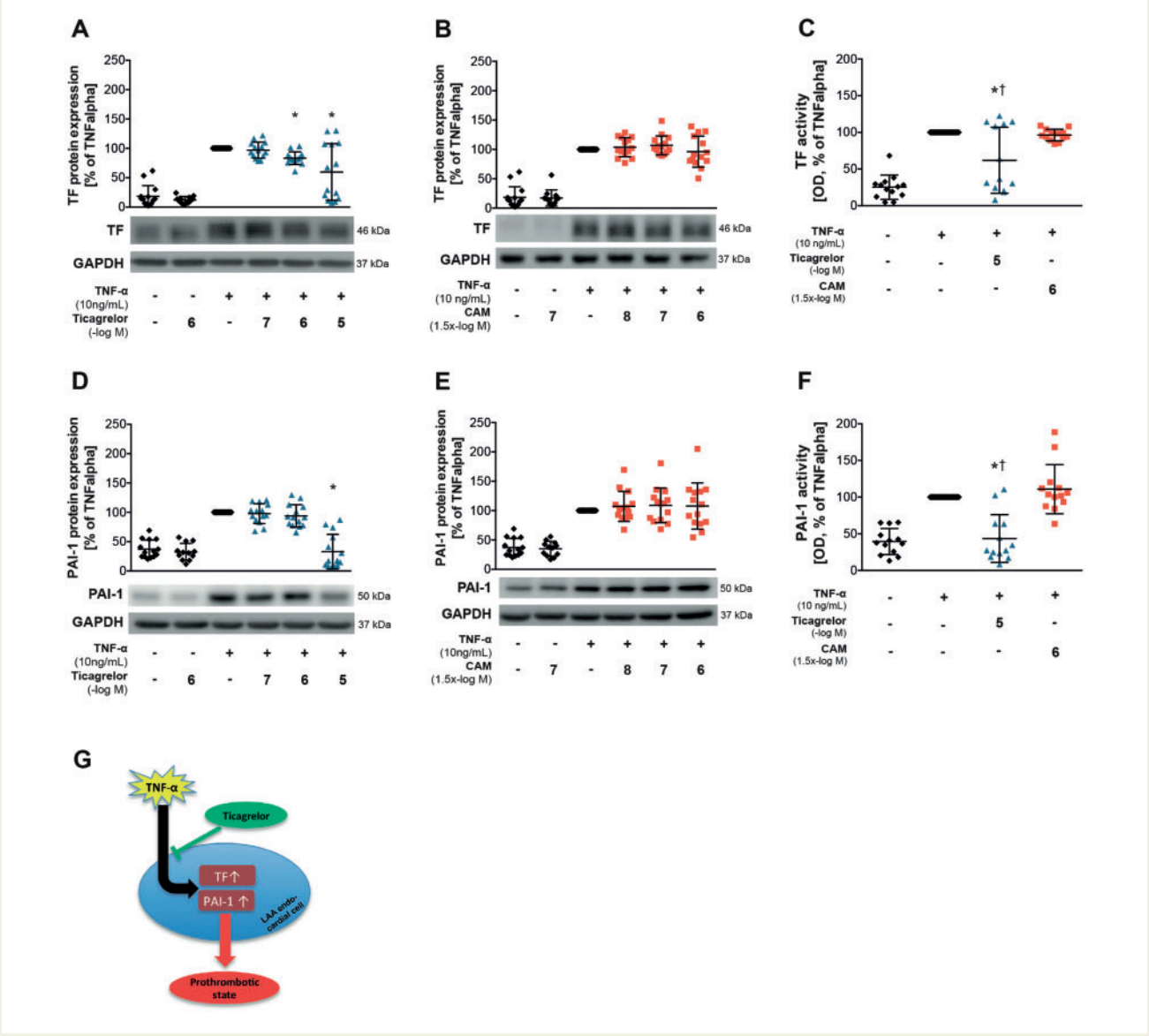

Figure 1 Effects of ticagrelor and CAM on expression and activity of thrombotic mediators in LAA endocardial cells. (*A*) Western blotting analysis for TF protein expression in LAA endocardial cells pretreated with increasing concentrations of ticagrelor ($n = 14$), or (*B*) CAM ($n = 14$) with/without TNF-α. (*C*) ELISA for TF activity in LAA endocardial cells pretreated with ticagrelor or CAM with/without TNF-α stimulation ($n = 13$). (*D*) Western blotting analysis for PAI-1 protein expression in LAA endocardial cells pretreated with increasing concentrations of ticagrelor ($n = 14$), or (*E*) CAM ($n = 14$) with/without TNF-α. (*F*) ELISA for PAI-1 activity in LAA endocardial cells pretreated with ticagrelor or CAM with/without TNF-α stimulation ($n = 13$). (*G*) Schematic representation of main study finding. Data are expressed as mean ± SD. One-way ANOVA with Tukey *post hoc* test or unpaired two-tailed Student's *t*-test was applied. **P* <0.05 vs. TNF-α; †*P* <0.05 vs. TNF-α + CAM. CAM, clopidogrel active metabolite; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LAA, left atrial appendage; PAI-1, plasminogen activator inhibitor-1; SD, standard deviation; TF, tissue factor; TFPI, tissue factor pathway inhibitor, TNF-α, tumour necrosis factor-alpha.

AF leads to thrombus formation in the left atrial appendage (LAA) due to a reduction in blood flow, endocardial activation, and hypercoagulability mediated through cytokines such as tumour necrosis factor-alpha (TNF-α).[4] We previously demonstrated that TNF-α-activated human LAA endocardial cells increase their levels of tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) expression and activity, which may contribute to a higher thrombogenicity in left vs. right atrial appendages.[5] An increasing body of evidence is suggesting pleotropic effects of certain P2Y12 receptor antagonists;[6,7] indeed, ticagrelor was shown to inhibit the adenosine transporter equilibrative nucleoside transporter 1,[6,7] leading to increased plasma levels of adenosine.[8] In the current study, we investigated whether ticagrelor or clopidogrel active metabolite (CAM) affect the expression of

prothrombotic mediators in TNF-α-activated human LAA endocardial cells.

Results

Ticagrelor, but not CAM, reduces TF expression and activity

Ticagrelor concentration dependently decreased TNF-α-induced TF expression in LAA endocardial cells (*Figure 1A*). The maximum effect amounted to 40% reduction in TF expression as compared with TNF-α stimulated cells. In contrast, CAM did not affect TF expression (*Figure 1B*). In line with protein expression, ticagrelor, but not CAM, reduced TF activity (*Figure 1C*). In contrast, endocardial protein expression of TFPI, the physiological antagonist of TF, was affected neither by ticagrelor nor CAM (see Supplementary material online, *Figure S1*).

Ticagrelor, unlike CAM, decreases PAI-1 expression and activity

Similar to TF, ticagrelor also reduced PAI-1 expression in TNF-α-stimulated LAA endocardial cells (*Figure 1D*). The maximum effect observed was a 67% reduction in PAI-1 expression as compared with TNF-α stimulated cells. In contrast, no effect on PAI-1 expression was observed with CAM (*Figure 1E*). Similarly, ticagrelor, but not CAM reduced enzymatic PAI-1 activity in LAA endocardial cells (*Figure 1F*).

Discussion

In this study, we demonstrate for the first time that ticagrelor, but not CAM, reduces TNF-α-induced TF and PAI-1 expression and activity in LAA endocardial cells isolated from patients with AF, hinting towards a possible local antithrombotic effect of ticagrelor at the cellular level.

Current treatment strategies for stroke prevention in patients with AF focus on inhibition of clot formation through interference with the coagulation cascade using vitamin K antagonists or non-vitamin K oral anticoagulants.[2,9,10] Antiplatelet therapy, even dual anti-platelet therapy using aspirin and clopidogrel, has been shown to be inferior to oral anticoagulation in stroke prevention in AF patients.[3,11] Newer generation antiplatelet agents such as ticagrelor, however, have not been evaluated for this indication.

Our current findings unravel a putative additional property of ticagrelor via which thromboembolic complications may be reduced, i.e. by decreasing local thrombogenicity of LAA endocardial cells through the reduction of crucial prothrombotic mediators such as TF and PAI-1. An increasing body of evidence suggests pleiotropic effects of ticagrelor;[6,7] indeed, it was shown to inhibit the adenosine transporter equilibrative nucleoside transporter 1,[6,7] thereby leading to increased adenosine plasma concentrations in cardiac patients.[8] The current results indicate the possibility of a local antithrombotic effect of ticagrelor in the LAA of AF patients. It currently remains unknown whether other anticoagulant drugs may have similar properties. A recent study reported that rivaroxaban, in contrast to dabigatran, reduced TF expression in endothelial cells.[12] However, these experiments were performed in human vein endothelial cells (instead of left atrial endocardial cells in our setting) and only reported changes in TF gene expression leaving out protein expression as well as enzyme activity.[12] Yet, these findings in congregate raise the possibility that local antithrombotic effects may be operative in the reduction of stroke risk observed with anticoagulants.

Twenty to 30% of patients with AF suffer from comorbidities such as coronary artery disease requiring antiplatelet therapy.[2,9] Optimal antithrombotic regimen in these patients is currently a matter of debate due to the high bleeding risk associated with combined anticoagulant and antiplatelet therapy.[9] While the role of new P2Y12 antagonists such as ticagrelor has not yet been addressed[9] and is subject of ongoing studies,[13] local antithrombotic mechanisms by ticagrelor in the LAA may be of particular interest (and value) in this specific patient population. Further studies are required to investigate whether the observed *ex vivo* effects of ticagrelor translate into improved patient outcome, including both thromboembolic as well as bleeding complications.

In summary, our results indicate that ticagrelor reduces the expression and activity of local procoagulant proteins in LAA endocardial cells, which may contribute to a reduction of thromboembolic complications in patients with AF. Our findings may instigate further research investigating the clinical implications of these findings, particularly for patients with AF in need of concomitant antiplatelet therapy.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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References

1. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, Gillum RF, Kim YH, McAnulty JH Jr, Zheng ZJ, Forouzanfar MH, Naghavi M, Mensah GA, Ezzati M, Murray CJ. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. *Circulation* 2014;**129**:837–847.

2. Camm AJ, Lip GY, De Caterina R Savelieva, I Atar, D Hohnloser, SH Hindricks, G Kirchhof, P. Guidelines ESCCfP. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation. Developed with the special contribution of the European Heart Rhythm Association. *Eur Heart J* 2012;**33**:2719–2747.
3. Connolly S, Pogue J, Hart R, Pfeffer M, Hohnloser S, Chrolavicius S, Pfeffer M, Hohnloser S, Yusuf S. Clopidogrel plus aspirin versus oral anticoagulation for atrial fibrillation in the Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events (ACTIVE W): a randomised controlled trial. *Lancet* 2006;**367**:1903–1912.
4. Watson T, Shantsila E, Lip GY. Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited. *Lancet* 2009;**373**:155–166.
5. Breitenstein A, Glanzmann M, Falk V, Maisano F, Stampfli SF, Holy EW, Finlay M, Ling LH, Schilling RJ, Luscher TF, Steffel J, Camici GG. Increased prothrombotic profile in the left atrial appendage of atrial fibrillation patients. *Int J Cardiol* 2015;**185**:250–255.
6. Armstrong D, Summers C, Ewart L, Nylander S, Sidaway JE, van Giezen JJ. Characterization of the adenosine pharmacology of ticagrelor reveals therapeutically relevant inhibition of equilibrative nucleoside transporter 1. *J Cardiovasc Pharmacol Ther* 2014;**19**:209–219.
7. Nylander S, Femia EA, Scavone M, Berntsson P, Asztely AK, Nelander K, Lofgren L, Nilsson RG, Cattaneo M. Ticagrelor inhibits human platelet aggregation via adenosine in addition to P2Y12 antagonism. *J Thromb Haemost: JTH* 2013;**11**:1867–1876.
8. Bonello L, Laine M, Kipson N, Mancini J, Helal O, Fromonot J, Gariboldi V, Condo J, Thuny F, Frere C, Camoin-Jau L, Paganelli F, Dignat-George F, Guieu R. Ticagrelor increases adenosine plasma concentration in patients with an acute coronary syndrome. *J Am Coll Cardiol* 2014;**63**:872–877.
9. Lip GY, Windecker S, Huber K, Kirchhof P, Marin F, Ten Berg JM, Haeusler KG, Boriani G, Capodanno D, Gilard M, Zeymer U, Lane D, Document R, Storey RF, Bueno H, Collet JP, Fauchier L, Halvorsen S, Lettino M, Morais J, Mueller C, Potpara TS, Rasmussen LH, Rubboli A, Tamargo J, Valgimigli M, Zamorano JL. Management of antithrombotic therapy in atrial fibrillation patients presenting with acute coronary syndrome and/or undergoing percutaneous coronary or valve interventions: a joint consensus document of the European Society of Cardiology Working Group on Thrombosis, European Heart Rhythm Association (EHRA), European Association of Percutaneous Cardiovascular Interventions (EAPCI) and European Association of Acute Cardiac Care (ACCA) endorsed by the Heart Rhythm Society (HRS) and Asia-Pacific Heart Rhythm Society (APHRS). *Eur Heart J* 2014;**35**:3155–3179.
10. Steffel J, Braunwald E. Novel oral anticoagulants: focus on stroke prevention and treatment of venous thrombo-embolism. *Eur Heart J* 2011;**32**:1968–1976,1976a.
11. Connolly SJ, Eikelboom J, Joyner C, Diener HC, Hart R, Golitsyn S, Flaker G, Avezum A, Hohnloser SH, Diaz R, Talajic M, Zhu J, Pais P, Budaj A, Parkhomenko A, Jansky P, Commerford P, Tan RS, Sim KH, Lewis BS, Van Mieghem W, Lip GY, Kim JH, Lanas-Zanetti F, Gonzalez-Hermosillo A, Dans AL, Munawar M, O'Donnell M, Lawrence J, Lewis G, Afzal R, Yusuf S, Committee AS Investigators. Apixaban in patients with atrial fibrillation. *N Engl J Med* 2011;**364**:806–817.
12. Ellinghaus P, Perzborn E, Hauenschild P, Gerdes C, Heitmeier S, Visser M, Summer H, Laux V. Expression of pro-inflammatory genes in human endothelial cells: comparison of rivaroxaban and dabigatran. *Thromb Res* 2016;**142**:44–51.
13. Gao F, Shen H, Wang ZJ, Yang SW, Liu XL, Zhou YJ. Rationale and design of the RT-AF study: combination of rivaroxaban and ticagrelor in patients with atrial fibrillation and coronary artery disease undergoing percutaneous coronary intervention. *Contemp Clin Trials* 2015;**43**:129–132.