Cystic fibrosis transmembrane conductance regulator potentiators attenuate platelet-activation and -aggregation in blood of healthy donors and COVID-19 patients

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a Cl^- channel and ABC transporter; its mutations cause the clinical picture of cystic fibrosis (CF). Of late, CFTR has also emerged as an important regulator of platelet function, as CFTR dysfunction causes agonist-induced platelet hyperactivation. These findings are reminiscent of platelets from SARS-CoV-2 infected patients since thromboembolic complications represent hallmarks of severe COVID-19 that may critically contribute to morbidity and mortality. Moreover, CFTR modulators have recently been introduced as a treatment for patients with various CFTR mutations, but have also been reported by us and others to enhance channel function of wild type CFTR. We therefore postulated that CFTR modulators may exert anti-coagulant effects on platelets, and may as such present a promising strategy to prevent thromboembolic complications in COVID-19.

We recruited 36 COVID-19 patients with moderate, and 34 COVID-19 patients with severe disease course (all w/o anti-platelet drugs), and 38 healthy donors (HDs). To determine the activation status of platelets, changes in surface expression of CD62p and CD63 upon pre-treatment with vehicle or the CFTR-potentiator ivacaftor were analyzed by flow cytometry. Platelets were activated by platelet-agonist adenosine diphosphate (ADP) or thrombin receptor activating protein-6 (TRAP6), and subsequently analyzed for Ca^{2+}-mobilization by FACS of INDO-1 labeled platelets, by impedance aggregometry, and by a microfluidic flow assay assessing platelet adhesion after pre-treatment with or without ivacaftor.

In line with our hypothesis, we observed significant reductions in ADP- or TRAP6-induced CD62p/CD63 expression, Ca^{2+}-mobilization, aggregation, and adhesion of platelets from HDs by pre-treatment with ivacaftor. In blood from COVID-19 patients, platelet-activation correlates with disease severity, as demonstrated by a 5-fold and 8-fold increase in the proportion of $CD62p^+$ platelets from patients with moderate and severe disease, respectively, relative to HDs. Similarly, the proportion of $CD63^+$ platelets in patients with severe COVID-19 was 2-fold higher than in HDs. Retrospective analysis of clinical data (*TriNetX* Real-World Database) of a total of 3.952 CF-patients with COVID-19 receiving single- or combination-therapy of ivacaftor, lumacaftor, tezacaftor, or elexacaftor in comparison to an untreated cohort revealed that the relative risk of suffering thromboembolism-associated cardiovascular events such as heart attack or deep vein thrombosis was reduced by 77.1% or 57.1%, respectively, suggesting an anti-thrombotic effect of CFTR modulators in CF^+/COVID-19 patients. In line with this observation, *ex vivo* pre-treatment of platelets from acute COVID-19 patients with ivacaftor reduced Ca^{2+} mobilization, adhesion, and aggregation of platelets.

Our results demonstrate an anticoagulant effect of CFTR potentiators on platelets from HDs and severe COVID-19 patients and suggest CFTR potentiators as a promising strategy to reduce the risk of thrombotic events in the clinical management of COVID-19.