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DURING ANTI-PLATELET THERAPY PLATELET TURNOVER MAY LEAD TO THE EMERGENCE OF A MINORITY OF UNINHIBITED PLATELETS SUFFICIENT TO INITIATE AND DRIVE PLATELET AGGREGATE FORMATION

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Introduction Patients with acute coronary syndromes, characterised by increased risk for atherothrombotic events, often receive ADP-$P2Y_{12}$ inhibitors such as clopidogrel or prasugrel as dual anti-platelet therapy together with aspirin, a cyclooxygenase inhibitor. Although short-lived, these drugs act irreversibly upon their targets and so are used as once a day treatments. The daily platelet turnover in healthy humans is approximately ten to fifteen per cent but can be considerably increased in disease conditions such as diabetes and chronic kidney disease. This leads to the daily emergence of an uninhibited subpopulation among the larger population of inhibited platelets. Here we investigated the role and contribution of this minority of uninhibited platelets in platelet aggregation.

Methods Human platelet rich plasma was incubated with 30 μM aspirin, 3 μM prasugrel active metabolite, or vehicle. Inhibited and uninhibited platelets were labelled with either green or red dye to distinguish between inhibited and uninhibited platelets once platelet populations were recombined. The responses of mixed populations of inhibited and uninhibited platelets to 20 μM ADP, 250 μM arachidonic acid (AA), 0.3 μg/ml collagen related peptide or 2 mg/ml ristocetin were followed by light transmission aggregometry, after which platelet aggregates formed were fixed and analysed by confocal microscopy and Imaris.

Results Platelet aggregation responses and images of platelet aggregates demonstrated complex, dynamic relationships between platelet subpopulations. Notably for treatment with $P2Y_{12}$ receptor blocker, aggregates demonstrated inhibited platelets surrounding central clusters of uninhibited platelets. Quantification demonstrated that aggregates formed in response to ADP had 82±6% clustering of uninhibited platelets at the core.

Discussion The existence of uninhibited platelet clusters within the centre of platelet aggregates indicates that a small minority of

uninhibited platelets can be a focal point for the recruitment of $P2Y_{12}$-inhibited platelets and the consequent formation of larger platelet aggregates. This is the first description of the roles of differently inhibited platelets in the formation of platelet aggregates and may well provide an explanation for the failure of dual anti-platelet therapy, particularly in patients with conditions associated with elevated platelet turnover.