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Short Communication

CD39 May Impact Clopidrogrel Pharmacodynamics

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Thrombosis is treated with antithrombotic drugs such as clopidogrel. Clopidogrel which is widely prescribed after heart attacks is a pro-drug that must be metabolically activated to the form that irreversibly blocks platelet $P2Y_{12}$ receptors. Bouman *et al.* [1] lately showed that the esterase paraoxonase-1 (PON1) was an enzyme involved in the catabolism of clopidogrel. Importantly, they showed that a common *PON1*gene polymorphismentails variability in clopidogrel's clinical efficacy, a factor thought to be a major drawback to its use. This implies that *PON1* genotyping might identify subjects unlikely to benefit from clopidogrel treatment. In agreement, E.Dolgin [2] described a case history of clopidogrel resistance correlating with low PON1 activity in a patient harbouring this genetic variant [3,4]. Taubert *et al.* [4] demonstrated that the $P2Y_{12}$ antagonist is generated by the action of 2 enzymatic steps performed by cytochrome P450 (CYP2C19) and by PON1. They also showed that the latter is the rate-limiting step responsible for clopidogrel resistance. This explanation was confirmed by Homes MV *et al.* [5] in an extensive study, on over 42,000 patients, where no clinically significant interaction of *CYP2C19* genotype with the association of clopidogrel therapy and cardiovascular events was shown.

The prognosis of patients on clopidogrel therapy that possess poor PON1 activity [1] might in fact be worse than expected. Indeed, Lecka *et al.* demonstrated that clopidogrel inhibits NTPDase1 activity [6]. NTPDase1 is responsible for ADP clearance from the blood and protects from uncontrolled platelet aggregation [7]. Indeed ADP is an important agonist of platelet activation and aggregation that is the natural ligand of $P2Y_{12}$ and also of $P2Y_1$, both expressed on platelets and responsible for their aggregation [7,8]. An oral dose of 30 mg clopidogrel, which constitutes 5% of the actual recommended therapeutic dosage, is expected to peak in the blood at ~20 µM. At this concentration, clopidogrel inhibited NTPDase1 activity, with either ATP or ADP as a substrate (>60% inhibition of ADPase activity). As a result, such inhibition favoured platelet aggregation *in vitro* [6]. These data are in agreement with the increased thrombosis and fibrin deposition found in *cd39*$^{+/-}$ mice in which the total enzyme activity of NTPDase1 (aka CD39) is reduced by half [7,9]. Conditions that diminish NTPDase1 activity have also been correlated with increased thrombosis and inflammation in several models [9-11].

Therefore, the previous comment suggesting that clopidogrel and its intermediary metabolites are "inactive" [12], on the assumption

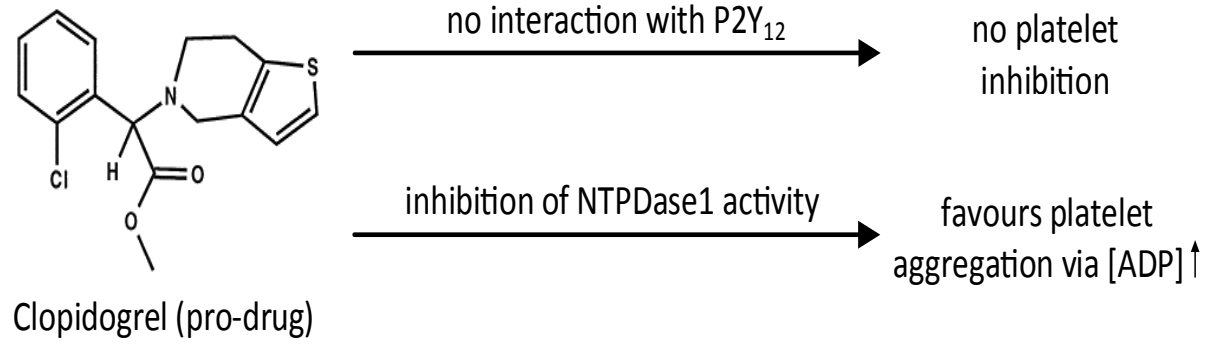

Figure 1: Schematic presentation of Topol and Schork's comment as it appeared under "News & Views" [11] in relation with the article by Bouman *et al.* [1], shows that clopidogrel, in its pro-drug form, does not inhibit $P2Y_{12}$ receptors and therefore does not block platelet aggregation (*upper* arrow). In a recent study [6] Lecka *et al.* showed that clopidogrel, at least in its pro-drug form, favours platelet aggregation via the build-up of ADP concentration at the endothelial cell surface due to the inhibition of NTPDase1 activity. Note that ADP is the natural ligand of $P2Y_1$ and $P2Y_{12}$ receptors and that by activating these receptors on platelet it plays a central role in regulating platelet function [7,8].

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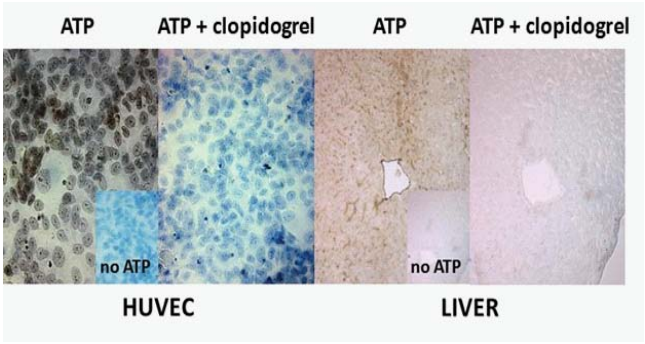

Figure 2: ATP hydrolysis at the surface of endothelial cells is blocked by clopidogrel. Freshly dissected thin sections of liver were embedded in O.C.T. freezing medium (Tissue-Tek®, Sakura Finetk, USA) and snap-frozen in isopentane in dry ice and stored at -80°C until use. Sections of 6 µm were prepared and fixed in 10% phosphate-buffered formalin mixed with cold acetone. Ectonucleotidase activity was located with the Wachstein-Meisel $Pb(NO_3)_2$ method [13]. Briefly, human liver sections or HUVEC grown on coverslips were fixed in 10% formalin in cold acetone, and further preincubated for 30 min at RT in 50 mM Tris-maleate buffer (pH 7.4) containing 2 mM $CaCl_2$, 250 mM sucrose and 2.5 mM levamisole as an inhibitor of alkaline phosphatase. The enzymatic reaction was performed for 1 h at 37°C in the same buffer supplemented with 5 mM $MnCl_2$, 2 mM $Pb(NO_3)_2$, 3% dextran T-250 ± 100 µM clopidogrel plus 200 µM ATP as substrate. For control experiments, the substrate was omitted. Reaction products were visualised by incubating the mixture with 1% $(NH_4)_2S$ v/v for 1 min. These sections were counterstained with aqueous haematoxylin and mounted in Mowiol mounting medium and photographed under a BX51 Olympus microscope. Positive ATPase activity was detected at the surface of cultured HUVEC as well as in liver blood vessels. No reaction was found in the absence of ATP (no ATP, *inset*) or in the presence of clopidogrel. Scale *bar*, 50 nm.

that they are not antagonists of $P2Y_{12}$ receptors, may reveal to be wrong. The study by Lecka *et al.* [6] rather suggests that in the absence of the active metabolite of clopidogrel, the "$P2Y_{12}$ inactive" clopidogrel pro-drug might in fact favour thrombosis by decreasing NTPDase1 activity (Figure 1). This effect, which might be increased and/or prolonged in patients where the catabolism of clopidogrel to its active form is impaired, such as in subjects with low PON1 and/or CYP2C19 activity [2-4,12], would be expected to precede the therapeutic action of clopidogrel (i.e. dependent on drug metabolism). In agreement with this observation [6], we show here that 100 µM clopidogrel blocks the hydrolysis of ATP by endothelial NTPDase1 at the surface of both human umbilical vein endothelial cells (HUVEC) and also at the surface of blood vessels in liver and pancreas tissues (Figure 2) and data not shown.

In the light of this interpretation, patients who cannot appropriately convert clopidogrel to a $P2Y_{12}$ antagonist, as in those exhibiting low PON1 and/or cytochrome 2C19 activity, might thus experience further complications from a therapy with this thienopyridine. Therefore, the addition of another antiplatelet drug together with clopidogrel, as is generally done, might be essential to ensure that NTPDase1 inhibition, albeit not yet tested *in vivo*, does not pose a threat for patientsduring clopidogrel therapy.

Keywords

NTPDase1; clopidogrel; pro-drug; thrombosis; PON1; $P2Y_{12}$

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