Inhibiting thrombosis without causing bleeding: can EP3 blockers fulfil the dream?

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This editorial refers to ‘Blocking the EP3 receptor for PGE2 with DG-041 decreases thrombosis without impairing haemostatic competence’ by P. Tilly et al., pp. 482–491, this issue.

Pharmacologists designing and clinicians dealing with antithrombotic drugs have always faced the problem that preventing thrombosis—by inhibiting one or more of the haemostatic pathways—always entails increased risk of bleeding. This paradigm has been over and over confirmed with the newer antithrombotic drugs, either inhibiting platelet function—such as prasugrel, ticagrelor, or cangrelor1—or inhibiting coagulation—the new direct oral anticoagulants, such as thrombin or activated Factor X inhibitors.2

No matter which new drug has been tested compared with older drugs or placebo, here the issue at stake is never increased potency or better safety in isolation, but always a better benefit–risk balance. Contrary to, for example, drugs lowering low-density lipoprotein (LDL) cholesterol, antithrombotic treatments have always been a perilous navigation between the Scylla of thrombosis and the Charybdis of bleeding. This latter itself also conjures with thrombosis because of the ominous consequences of bleeding, itself often precipitating thrombosis for a variety of reasons.3 Therefore, always protection from thrombosis has come to the expenses of increased bleeding—no free lunch.

Tilly et al.4 are now going to re-ignite the hypes and the hopes for such a class of magic compounds. Here inhibiting in vivo the receptor EP3 for prostaglandin (PG) E2 with the blocking agent DG-041 reduced murine thrombosis triggered by local delivery of arachidonic acid (AA) or ferric chloride on healthy arteries. Such a treatment also reduced thrombosis triggered by scratching murine atherosclerotic plaques. Blocking EP3 did not alter murine tail, liver, or cerebral haemostasis. Furthermore, blocking EP3 reduced murine pulmonary embolism and intensified platelet inhibition by clopidogrel, leaving tail bleeding times unchanged. In healthy humans, DG-041 reduced platelet aggregation, but did not significantly alter the cutaneous bleeding time at doses up to eight times higher than the dose that inhibited the facilitating effect of PGE2 on platelets.4

Can such a class of compounds harness the holy grail of preventing or inhibiting thrombosis without causing increased bleeding? To put these findings in perspective, I will first describe the rationale for developing such a compound in this direction; then discuss the potential consequences of EP3 inhibition; and finally discuss the possible theoretical limitations of such an approach.

The actions of PGE2 are determined by the distribution and activity of its receptors, termed endoperoxide–prostaglandin (EP) receptors. The diversity of action of PGE2 is explained both by its sites of production and by the different action specificity and tissue distribution of such receptors. There are four known PGE2 receptors, all belonging to the family of cell surface, G protein-coupled, seven transmembrane domain receptors, and known as EP1-(PGE2) (PTGER1), EP2-(PGE2) (PTGER2); EP3-(PGE2) (PTGER3); and EP4-(PGE2) (PTGER4).2,5,6 In particular, EP3, which has multiple alternatively spliced transcript variants encoding eight distinct isoforms (http://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=5733), may have many biological functions, which involve digestive, nervous system, kidney...
reabsorption, and uterine contraction activities (Figure 2). In the stomach, stimulation of EP3 inhibits gastric acid secretions. Studies of the mouse counterpart suggest that this receptor may also mediate adrenocorticotropic hormone response, as well as fever generation in response to exogenous and endogenous stimuli.

Platelets feature EP2, EP3, and EP4 PGE2 receptors.7,8 Of these, EP3 inhibits,7,9 while EP2 and EP4 appear to activate10–12 adenylate cyclase. Both because of the higher affinity of PGE2 for EP3,13 and because of EP3 predominant activity,7,9 the EP3-induced inhibition of adenylate cyclase predominates over EP2 and EP4 activation, which activate adenylate cyclase. So, PGE2 globally decreases the intraplatelet production of cyclic AMP (cAMP), which itself would inhibit calcium mobilization occurring after exposure of platelets to conventional platelet activators such as adenosine diphosphate (ADP), collagen, thrombin, or thromboxane (TX) A2. Therefore, overall, PGE2 increases platelet responses, i.e. sensitizes platelets to its activators and rescues the function of P2Y12-blocked platelets, while alone it does not induce platelet aggregation. Specific inactivation of EP3, for example with the compound DG-041 used in the protocol (Figure 1).
study by Tilly et al.\textsuperscript{,4} synergizes with activation of EP2 and EP4 receptors by PGE\textsubscript{2} to increase the amount of intraplatelet cAMP, resulting in enhanced inhibition of platelet response and inhibition of thrombosis. Consistent with this, the authors had previously reported that in vivo murine atherothrombosis was drastically reduced by the lack of EP3 on platelets.\textsuperscript{15} Not all the literature is however consistent on such findings: indeed, selective knock-out of the EP3 gene had shown increased bleeding,\textsuperscript{7} and the very same impact of PGE\textsubscript{2} on human platelets has been questioned by other reports.\textsuperscript{16,17}

Irrespective of previous findings, however, Tilly et al. here demonstrate that inhibiting the receptor EP3 for PGE\textsubscript{2} in vivo with the blocking agent DG-041 (a) reduced thrombosis in three murine models; (b) reduced murine pulmonary embolism; and (c) intensified platelet inhibition by clopidogrel, and all this without altering murine tail, liver, or cerebral haemostasis.\textsuperscript{4} This supports the original hypothesis by the authors, leaving hope for the further development of this class of compounds as novel, useful antplatelet agents, potentially different from compounds currently available.

Reservations still however linger on the final viability of this potential strategy for a variety of reasons.

First, despite blocking platelet function only in conditions of PGE\textsubscript{2} production, such as in atherothrombosis, inhibited platelets would still circulate, and those inhibited platelets, in which the sensitivity to P2Y\textsubscript{12} receptor blockers such as clopidogrel is enhanced, can theoretically still lead to bleeding at remote injury sites in the same subject. Therefore, once more, the lack of effect on normal haemostasis would still need further confirmation.

Secondly, the production of the primary agonist, PGE\textsubscript{2}, considered for targeting its receptor-mediated effects, is itself susceptible to inhibition by COX inhibitors, including high-dose aspirin, conventional non-steroidal anti-inflammatory drugs, and coxibs. High-dose aspirin has never been shown of superior efficacy compared with low-dose aspirin, selectively targeting platelet COX-1 and platelet TX production.\textsuperscript{18} Of course, one can attribute this lack of increased efficacy, or even the adverse effects on thrombosis by treatment with coxibs to the curtailing of prostacyclin (PGI\textsubscript{2}) production by such compounds,\textsuperscript{19} but such strategies would also have had the result of curtailing the local concentrations of the agonist for the EP3 receptor, with some end results expected to be similar to those of EP3 inhibition. In addition, would the antiplatelet effects of this new class of compound be abrogated in patients treated with high-dose aspirin or non-steroidal anti-inflammatory drugs, or coxibs?

Thirdly, the ubiquitous nature of EP3\textsuperscript{5} makes it unlikely that compounds such as DG-041 only have effects on platelets, and untowards...
effects in other organs or systems may ultimately render this approach unviable.

Thus, the forecast is that the road to the development of such compounds as useful antithrombotic agents will not be without dangers. The history of antiplatelet agents is itself a source of teaching and warning on the development of compounds originally planned to be devoid of bleeding problems. Such were, for example, the development of dipyridamole and of the protease-activated receptor (PAR)-1 antagonist vorapaxar, both born with such highly emphasized character-


