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

Raffaele De Caterina*

Institute of Cardiology and Center of Excellence on Aging, ‘G. D’Annunzio’ University, Chieti, and G. Monasterio Foundation, Pisa, Italy

Online publish-ahead-of-print 26 January 2014

This editorial refers to ‘Blocking the EP1 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 cangrelor—or inhibiting coagulation—the new direct anticoagulants, such as thrombin or activated Factor X inhibitors. 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. Therefore, always protection from thrombosis has come to the expenses of increased bleeding—no free lunch.

Tilly et al. 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.

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.

PGE2 is one of the myriad of compounds (eicosanoids) produced through the metabolism of the n-6 polyunsaturated fatty acid arachidonate (AA), largely esterified in the sn2 position of membrane phospholipids (Figure 1). Once liberated through the action of phospholipases (PL, mainly PLA2), one pathway of metabolism of AA is through cyclooxygenase (COX) 1 and 2. COX metabolism has been the first pathway of AA to be discovered. The isomerase catalyzing the conversion of the unstable intermediates PGG2/PGH2 into PGE2 is called PGE synthase. Humans express three PGE synthase isozymes, each encoded by a separate gene. Out of the three isozymes, PGES1 and 2 can be found in the microsomal fraction and PGES3 in the cytoplasm of inflammatory cells, including neutrophils and macrophages, but also smooth muscle cells, the kidney, the placenta, and several cancer cells. The corresponding genes are inducible by inflammatory mediators, such as interleukin-1β and tumour necrosis factor-α. These findings explain why, contrary to normal vessels, human atherosclerotic plaques, which contain inflammatory cells—mostly monocyte-derived macrophages—produce PGE2.

PGE2 was discovered by Bunting, Gryglewski, Moncada, and Vane back in 1976, has important effects in labour (it softens the cervix and causes uterine contraction), and also stimulates osteoblasts to release factors that stimulate bone resorption by osteoclasts (its up-regulation is implicated as a possible aetiology of nail clubbing). PGE2 is also the prostaglandin that ultimately induces fever. It is a direct vasodilator, relaxing smooth muscles, and inhibits the release of noradrenaline from sympathetic nerve terminals. It is also implicated in duct-dependent congenital heart diseases and is used in infusion to keep the duct open in such diseased newborns.

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 EP2 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). 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

* Corresponding author. Tel: +39 0871 4151; fax: +39 0871 402817; Email: rdecater@unich.it

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2014. For permissions please email: journals.permissions@oup.com.
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

![Figure 1](https://academic.oup.com/cardiovascres/article-abstract/101/3/335/462604/fig1)
study by Tilly et al.,4 synergizes with activation of EP2 and EP4 receptors by PGE2 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.15 Not all the literature is however consistent on such findings: indeed, selective knock-out of the EP3 gene had shown increased bleeding,7 and the very same impact of PGE2 on human platelets has been questioned by other reports.16,17

Irrespective of previous findings, however, Tilly et al. here demonstrate that inhibiting the receptor EP3 for PGE2 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.4 This supports the original hypothesis by the authors, leaving hope for the further development of this class of compounds as novel, useful antiplatelet 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 PGE2 production, such as in atherothrombosis, inhibited platelets would still circulate, and those inhibited platelets, in which the sensitivity to P2Y12 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, PGE2, 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.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 (PGI2) production by such compounds,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 EP35 makes it unlikely that compounds such as DG-041 only have effects on platelets, and untowards

---

**Figure 2** A closer look of the action of prostaglandin (PG) E2 and its receptor (EP1–EP4), with a focus on the EP3 receptor. For abbreviations, see Figure 1. See text for further details.

[Diagram showing the action of prostaglandin E2 and its receptors EP1-EP4, with a focus on EP3, and the role of DG-041.]
effects in other organs or systems may ultimately render this approach unavailable.

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 characteristics, and ultimately found to have themselves the same, recurrent, apparently unavoidable Achilles’ heel of increased bleeding. Therefore, while we should welcome the testing of a new hypothesis for inhibiting thrombosis with a better efficacy—safety profile than with current drugs, we warn about premature enthusiasm and emphasize the need for further studies in this exciting field.

Conflict of interest: none declared.

References