Blocking the EP₃ receptor for PGE₂ with DG-041 decreases thrombosis without impairing haemostatic competence

Peggy Tilly¹†, Anne-Laure Charles¹,²†, Sophie Ludwig¹, Farid Slimani¹, Sabrina Gross¹, Olivier Meilhac³, Bernard Geny²,⁴, Kari Stefansson⁵, Mark E. Gurney⁵,⁶ and Jean-Etienne Fabre¹*

¹Institut de Génétique et de Biologie Moléculaire et Cellulaire, Institut National de la Santé et de la Recherche Scientifique UMR7104, Université Louis Pasteur, 67400 Illkirch, France; ²Université de Strasbourg, Fédération de Médecine Translationnelle, Équipe D’Accueil 3072, 67000 Cedex, France; ³Institut National de la Santé et de la Recherche Médicale U698, CHU X-Bichat, Paris, France; ⁴Service de Physiologie et d’Explorations Fonctionnelles, Pôle de Pathologie Thoracique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; ⁵deCODE Genetics, Sturlugata 8, IS-101, Reykjavik, Iceland; and ⁶Tetra Discovery Partners, 301 Michigan St NE, Grand Rapids, MI 49506, USA

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Aims

Haemostasis interrupts bleeding from disrupted blood vessels by activating platelet aggregation and coagulation. A similar mechanism termed thrombosis generates obstructive thrombi inside diseased arteries. As a consequence of this similarity, current anti-thrombotic agents increase the risk of bleeding. Atherosclerotic plaques produce significant amounts of prostaglandin E₂ (PGE₂), which activates its receptor EP₃ on platelets and aggravates atherothrombosis. We investigated whether blocking EP₃ could dissociate atherothrombosis from haemostasis.

Methods and results

Inhibiting in vivo the receptor EP₃ for PGE₂ with the blocking agent DG-041 reduced murine thrombosis triggered by local delivery of arachidonic acid or ferric chloride on healthy arteries. Importantly, it also reduced thrombosis triggered by scratching murine atherosclerotic plaques. PGE₂ was not produced at the bleeding site after tail clipping. Consistently, 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. Human atherosclerotic plaques produced PGE₂, which facilitated platelet aggregation in human blood and rescued the function of P2Y₁₂-blocked platelets. Finally, in healthy patients, DG-041 reduced platelet aggregation, but did not significantly alter the cutaneous bleeding time at doses up to eight times the dose that inhibited the facilitating effect of PGE₂ on platelets.

Conclusion

In mice, blocking EP₃ inhibited atherothrombosis without affecting haemostasis and intensified efficiency of conventional anti-platelet treatment without aggravating the bleeding risk. In patients, blocking EP₃ should improve the prevention of cardiovascular diseases, which is currently limited by the risk of bleeding.

Keywords

Atherothrombosis • Prostaglandins • Haemostasis • Anti-platelet agents

1. Introduction

Atherothrombosis, the leading cause of death worldwide, is partly prevented by current anti-platelet agents.¹ More potent drugs²,³ or drug combinations increase the risk of bleeding,⁴–⁶ and bleeding itself increases the risk of re-infarction and death.⁷ Thus, there is a need for drugs able to intensify the anti-platelet effect without altering haemostasis, although the molecular mechanisms at play in haemostasis and thrombosis are highly intertwined. Some pathways involved in coagulation or platelet aggregation have been shown to predominate in thrombosis over haemostasis with the promise to reduce thrombotic events without increasing the bleeding risk.³ Another strategy is to seek pro-thrombogenic factors that are produced in much higher amounts by atherosclerotic plaques than by healthy vascular wall.

¹ These two authors contributed equally to the study.
² Corresponding author. IGBMC, 1, rue L. Fries, 67404 Illkirch CEDEX, France. Tel: +33 3 88 65 33 85; fax: +33 3 88 65 32 01. Email: jeffabre@igbmc.fr

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Drugs targeting such factors could reduce atherothrombosis with a minor impact on haemostasis. Atherosclerotic plaques are inflammatory lesions in which the arachidonic acid (AA) pathway is activated, producing for instance prostaglandin E2 (PGE2), which activates its EP2, EP3, and EP4 specific receptors on platelets, whereas EP2 and EP4 activate the adenylate cyclase. Both higher affinity of PGE2 for EP3 and murine in vivo models of thrombosis showed that the EP3-inhibited induction of adenylate cyclase predominates over EP2 and EP4 activations. So, PGE2 globally decreases the intraplatelet production of cyclic AMP (cAMP), which itself inhibits calcium mobilization triggered by conventional platelet activators, such as adenosine diphosphate (ADP), collagen, thrombin, or thromboxane A2 (TXA2). Hence, PGE2 increases platelet response, i.e. sensitizes platelets to its activators while alone it does not induce platelet aggregation. Specific inactivation of EP3 synergizes with activation of EP2 and EP4 receptors by PGE2 to increase the amount of intraplatelet cAMP that inhibits the platelet response. Consistent with this, we previously reported that in vivo murine atherothrombosis was drastically reduced by the lack of EP3 on platelets.

We also previously observed that tail transection of EP3-deficient mice did not increase bleeding in mice. Since human plaques produce PGE2, these data suggest that targeting the PGE2/EP3 pathway might prevent atherothrombosis without altering haemostasis. However, the lack of impact on murine haemostasis has been challenged, the impact of PGE2 on human platelets has been questioned, and demonstration that targeting the PGE2/EP3 pathway dissociates thrombosis from haemostasis is lacking. To test the hypothesis that atherothrombosis can be controlled without inducing bleeding in mice, we used a selective EP3 blocker, known to inhibit PGE2-induced potentiation of aggregation of rat and human platelets.

Using several models, the pharmacological EP3 blockade reduced in vivo thrombosis, but did not alter haemostasis in several vascular beds. We showed that PGE2 re-sensitized platelets in which the ADP pathway was blocked by clopidogrel, and that associating DG-041 to clopidogrel allowed the anti-thrombotic effect to be intensified without aggravating the clopidogrel-induced bleeding. Furthermore, we have shown here that PGE2 was produced by human plaques, and that PGE2 facilitated platelet aggregation in human whole blood. Finally, DG-041 inhibited human platelet aggregation, but not human bleeding time. These data show that blocking the PGE2/EP3 pathway can reduce thrombosis without altering haemostasis.

2. Methods

2.1 Mice

Mouse procedures were approved by ethical committees (Strasbourg), conforming to the directive 2010/63/EU. Anaesthesia and euthanasia were performed with isoflurane. All experiments were performed by investigators blinded to treatments and/or genotypes (full methods in Supplementary material online).

2.2 Platelet aggregation tests

About 250 μL of platelet-rich plasma (PRP, 300 000 platelets/μL) isolated from citrated blood were used for aggregation tests; when indicated, platelets were incubated with DG-041 at 25°C for 10 min.

2.3 Quantification of murine thrombosis by intravital macroscopy

Left carotid arteries of mice injected with calcein (300 ng/mL)-loaded platelets were exposed to either 4 μL of AA (100 mg/mL, 1 min) or ferric chloride 4% (1 μL, 5 min). Fluorescent thrombosis was video-recorded through a MacroFluo (Leica).

2.4 Mouse model of atherothrombosis

Atherosclerotic plaques were scratched in blood flow using a tiny needle introduced through a branch of the external carotid artery (Figure 1).

2.5 Measurement of PGE2 at the mouse site of bleeding

After amputating 5 mm of murine tail tips, another 3 mm wide piece was cut off for PGE2 detection immediately vs. after bleeding cessation. PGE2 was detected by Enzyme ImmunoAssay (EIA).

2.6 Quantification of murine blood loss

After amputating 7 mm of murine tail tips, surging blood was collected in distilled water (30 mL) up to bleeding cessation. Blood volume was determined from the OD540 plotted against a calibration curve, corrected by blood haemoglobin.

2.7 Liver bleeding

In mice gavaged with vehicle, DG-041 (60 mg/kg) or clopidogrel (10 mg/kg), the median abdominal line was cut 1 h (water, DG-041) or 6 h (clopidogrel) after the gavage. A calibrated piece (3.90 ± 0.15 mg, n = 46) was chopped off from the inferior edge of the right lobe of the liver and the cavity closed. Red blood cells (RBCs) were counted in the 4 mL peritoneal lavage 20-min later.

2.8 Cerebral bleeding

A small borehole was drilled at 2 mm laterally to the bregma. A 25-G needle was lowered down to a 4 mm mark, rotated 360°, and removed. After euthanasia and transcardial perfusion 30-min later, the brain was extracted and cut (1 mm thickness). Haematoma was measured using Image J after deconvolution (fast red-fast blue-DAB; threshold: 160).

2.9 Determinations of murine bleeding time

To test DG-041 increasing doses, a transverse incision was made over the right lateral tail vein. Before AA-induced thrombosis, mice receiving clopidogrel or DG-041 had their 5 mm tail tips transected. In all these cases, the tail was immersed into phosphate buffered saline (37°C). Bleeding times were measured from incision or transection to first cessation of bleeding.

2.10 Model of mouse pulmonary thromboembolism

Thirty minutes after gavaging mice with DG-041, 5 mL/kg of a solution combining a TXA2 mimetic, U46619 (0.6 μmol/L), and the selective EP3 agonist sulprostone (0.05 mg/mL) was injected via a lateral tail vein to induce intravenous thrombosis through the PGE2/EP3 pathway. Survival was noted 60 min later.

2.11 Measurement of PGE2 in human atherosclerotic plaques

Human carotid endarterectomy samples and non-atherosclerotic endarteries were obtained from patients undergoing carotid surgery or coronary bypass. These tissues, considered as surgical waste, were incubated for 24 h in a volume of Roswell Park Memorial Institute medium adjusted to the wet weight (6 mL/g of tissue) before performing EIA.
2.12 Blood sample collection from human subjects
Informed consent was obtained from healthy volunteers (20–40 years old) in accordance with the Helsinki protocol from local bioethical committees (Strasbourg: CPP#09/504 or the National Bioethics Committee of Iceland). Fresh blood (venipuncture) was collected in citrated tubes from subjects not taking non-steroidal anti-inflammatory drugs.

2.13 Phosphorylated VAsodilator-Stimulated Phosphoprotein assay
Lysed platelets (radioimmunoprecipitation assay buffer) were used to measure protein concentration or phosphorylated VAsodilator-Stimulated Phosphoprotein (pVASP) by EIA. The EIA plates were coated with a monoclonal anti-VASP antibody (Immunoglobe); the primary antibody directed against pVASP Ser157 (Santa Cruz) was revealed by a Horse Radish Peroxidase-α rabbit secondary antibody (DakoCytomation).

2.14 DG-041-CV-007 human clinical trial
This single-blind, randomized, placebo-controlled study (Institutional Review Board at the clinical site) studied three doses of DG-041 (100, 400, and 800 mg twice a day for 7 days). Ten healthy volunteers (18–50 years old) were enrolled into each of the three cohorts. In each cohort, eight subjects were randomized to DG-041 and two to placebo. Bleeding times and platelet aggregation were measured at entry (pre-dose), and 4 h after the morning dose on the seventh day of treatment.

3. Results
3.1 Blocking the EP₃ receptor with DG-041 reduced murine atherothrombosis
Gavaging wild-type mice with DG-041 (60 mg/kg) dramatically inhibited the facilitating effect induced by PGE₂ (10.0 ± 4.8%, n = 7 vs. 60.4 ± 6.0% of maximal aggregation, n = 7 control mice), as efficiently as Ep3−/− platelets (see Supplementary material online, Figure S1).

![Figure 1](https://academic.oup.com/cardiovascres/article-abstract/101/3/482/460643)
Furthermore, DG-041 inhibited thrombosis in vivo induced by AA or ferric chloride superfused on carotid arteries (see Supplementary material online, Figure S2). Moreover, we scratched the luminal surface of atherosclerotic plaques in ApoE<sup>−/−</sup> carotid arteries to provoke atherothrombosis<sup>9</sup>. DG-041 strikingly reduced the fluorescent atherothrombosis (<span class="math" style="font-size:100%;">0.02 ± 0.01 × 10^5</span> pixels/min, <span class="math" style="font-size:100%;">n = 12</span>) observed in control mice (<span class="math" style="font-size:100%;">1.80 ± 0.60 × 10^5</span> pixels/min, <span class="math" style="font-size:100%;">n = 13</span>, Figure 1).

Thus, the selective blockade of EP<sub>3</sub> inhibited murine thrombosis and atherothrombosis.

### 3.2 The PGE<sub>2</sub>/EP<sub>3</sub> pathway was not involved in murine haemostasis

To examine the role of the PGE<sub>2</sub>/EP<sub>3</sub> pathway in haemostasis, we first examined whether PGE<sub>2</sub> is produced in response to injury. After the amputation of tail tips (5 mm), PGE<sub>2</sub> amounts detected in the next 3 mm distal part of the tails were similar in samples taken upon beginning (<span class="math" style="font-size:100%;">555 ± 72 pg/mg</span>, <span class="math" style="font-size:100%;">n = 5</span>) vs. cessation (<span class="math" style="font-size:100%;">580 ± 88 pg/mg</span>, <span class="math" style="font-size:100%;">n = 5</span>, Figure 2A) of bleeding. This lack of difference suggests that the cessation

Figure 2  Mouse haemostasis was not EP<sub>3</sub>-dependent. (A) Detection of PGE<sub>2</sub> in tail 3 mm tip after a first transection (5 mm) at beginning vs. end of bleeding. (B) Blood loss after tail tip transection in mice which underwent plaque scratching and received vehicle (HPbCD) or DG-041 and in age-matched sham-operated mice (WT). (C) Number of RBCs in peritoneal lavage after a calibrated injury of the liver in mice receiving water, DG-041 (60 mg/kg), or clopidogrel (10 mg/kg). Inset: macroscopic aspect of lavages collected from DG-041-treated (left) vs. clopidogrel-treated mice (right). (D) Photographs from brain slices of mice treated with DG-041 (60 mg/kg, left column) vs. clopidogrel (10 mg/kg, right column). Below: Graph showing the number of red pixels detected on brain slices in each group.
of bleeding did not require local production of PGE2. This basal amount of PGE2 detected in the tail tissue does not interfere with the platelet function, since amputation of the tail tips did change the volume of blood loss neither in Ep3+/− mice (see Supplementary material online, Figure S2) nor in Apoe−/− mice of which plaques in carotid arteries were scratched. In the latter, the volumes of lost blood were not significantly different in mice treated with vehicle (128 ± 30 μL, n = 10), DG-041 (85 ± 27 μL, n = 10), or in sham-operated and age-matched WT mice (163 ± 33 μL, n = 10, Figure 2B). We concluded that the PGE2/EP3 pathway was not significantly involved in tail haemostasis.

Since haemostasis is tissue-specific,26,27 we further explored the impact of EP3 blockade with DG-041 on liver and brain bleeding. After a calibrated piece of liver was chopped off from the inferior edge, the number of RBC in the peritoneal lavage did not change in mice gavaged with DG-041 (60 mg/kg) when compared with mice receiving water (1.04 ± 0.39 × 106 RBC/μL, n = 14 vs. 0.95 ± 0.18 × 106 RBC/μL, n = 15). Conversely, mice gavaged with 10 mg/kg clopidogrel lost significantly more blood (5.22 ± 0.97 × 105 RBC/μL, n = 17, Figure 2C). To investigate the effect of EP3 on brain haemostasis, we used a model of needle-induced intracerebral haemorrhage.28 The extent of the cerebral haematoma was significantly increased only in mice receiving 10 mg/kg clopidogrel (22086 ± 5475 red pixels, n = 10), not in DG-041 (60 mg/kg) or water-gavaged mice (6864 ± 1247 red pixels, n = 13 vs. 4967 ± 668 red pixels, n = 10, Figure 2D).

Thus, blocking the EP3 function did not alter tail, liver, or brain haemostasis.

3.3 High doses of DG-041 protected against pulmonary embolism, but maintained haemostasis

Next, we used a model of pulmonary thromboembolism to test whether haemostasis remains competent, although doses of DG-041 are increased. Almost all mice (86%) co-infused with sub-stimulating amounts of U46619 and sulprostone, an EP3 agonist, died. This percentage dropped to 20% (n = 15, Figure 3A—bottom) in the group of mice pre-treated with DG-041 at a dose of 10 mg/kg or greater. Conversely, bleeding induced by a calibrated transverse incision of the tail was unaltered by the EP3 antagonist (88 ± 7 μL, n = 27 receiving 100 mg/kg vs. 69 ± 6 μL, n = 26 controls, Figure 3A—top). This experiment which involved a strong EP3 stimulation clearly shows that increasing the doses of DG-041 decreased thrombosis, while haemostasis remained unchanged.

3.4 Activating the PGE2/EP3 pathway restored the sensitivity of murine P2Y12-blocked platelets

Since EP3 activation decreases the cAMP platelet content and hence increases platelet sensitivity, we examined whether PGE2 can re-sensitize platelets subjected to clopidogrel, which blocks the ADP receptor P2Y12 to prevent thrombosis.29 Platelets from mice gavaged with clopidogrel (5 mg/kg) and exposed to ADP (1 μmol/L) aggregated only in the presence of 10−6 M PGE2 (3.81 ± 0.69%, n = 10 vs. 0.40 ± 0.14%, n = 10; Figure 3B). Thus, PGE2 partially restored the sensitivity of clopidogrel-treated platelets to ADP. Therefore, blocking the PGE2/EP3 pathway should increase the anti-thrombotic effect induced by conventional platelet activators.

3.5 Blocking EP3 intensified platelet inhibition without exacerbating bleeding

We next used the model of AA-induced thrombosis to test whether DG-041 can intensify platelet inhibition induced by clopidogrel. Clopidogrel at 2.5 mg/kg reduced thrombosis, but also significantly increased the bleeding time from 120 ± 10 (controls, n = 9) to 301 ± 58 s (n = 13, Figure 3C—top). Adding DG-041 to clopidogrel further decreased thrombotic scores from 10.72 ± 2.46 × 105 (n = 14) to 3.12 ± 1.13 × 105 pixels/min (n = 13, Figure 3B—bottom), while the bleeding time did not significantly increase (279 ± 52 s, n = 15). To obtain a similar anti-thrombotic effect with clopidogrel alone, we had to increase the dose up to 10 mg/kg, which clearly exacerbated bleeding (463 ± 51 s, n = 13). Thus, blocking EP3 intensified the platelet inhibition without worsening the bleeding induced by clopidogrel. This opens the possibility to dissociate thrombosis from haemostasis in vivo, at least in mice.

3.6 Human atherosclerotic plaques produced PGE2

To examine whether this possibility to control thrombosis independently from haemostasis is clinically relevant, we first checked whether human atherosclerotic plaques produce PGE2. Carotid plaques or fragments of non-atherosclerotic endarteries collected from patients were incubated in adequate culture medium. The 24-h cumulative amounts of PGE2 produced by stenotic (279 ± 48 pg/mg, n = 10) or non-stenotic (144 ± 23 pg/mg, n = 10) fragments of plaques were significantly higher than those yielded by fragments of non-atherosclerotic arteries (6.38 ± 2.12 pg/mg, n = 11, Figure 4A). Thus, human plaques produce PGE2.

3.7 The PGE2/EP3 pathway facilitated human platelet aggregation in whole blood

Since EP3 gene sequences30 and platelet expression10,11 are very close in mice and humans, platelet reactivity might be similar in both species. Indeed, PGE2 facilitates in vitro human platelet aggregation in PRP.31,32 To check whether PGE2 also facilitates platelet aggregation in whole blood, blood samples from nine healthy volunteers were subjected to the lowest dose of U46619 triggering a sub-maximal platelet aggregation (166 ± 15 mmol/L). The adjunction of PGE2 (10−7 mol/L) to this U46619 dose significantly increased the platelet aggregation (Figure 4B), showing that PGE2 also facilitated platelet aggregation in human whole blood.

3.8 Activating the PGE2/EP3 pathway restored the sensitivity of human P2Y12-blocked platelets

Next, we tested whether PGE2 can restore the function of human platelets inhibited by standard medications. We obtained platelets from patients with peripheral arterial disease and medicated with aspirin (80 mg/day, n = 6, ages 61–74) or clopidogrel (75 mg/day, n = 5, ages 48–91), and from non-medicated control subjects (n = 5, ages 66–71). VASP, a cytoskeleton protein, is phosphorylated by cAMP/cGMP kinases; pVASP is thus a marker of platelet cAMP levels.33,34 Thereby, the dephosphorylation of pVASP indicates a CAMP decrease resulting from platelet increased sensitivity or from platelet activation. As expected, adding ADP to ex vivo platelets dephosphorylated pVASP in platelets from control- or aspirin-treated patients, but not in those from clopidogrel-treated patients (93.6 ± 18.5 vs. 32.1 ± 4.8% in
controls, Figure 4C). In contrast, the EP$_3$ agonist sulprostone dephosphorylated pVASP in all platelet samples, including the group protected by clopidogrel (31.1 ± 4.9 vs. 29.9 ± 6.6% in controls). Thus, these human platelets subjected to standard oral anti-platelet treatments were re-sensitized by EP$_3$ activation. These results suggest that PGE$_2$ produced locally by the plaque can increase the sensitivity of platelets to their activators, even in patients receiving anti-platelet therapy.

3.9 Blocking EP$_3$ on human platelets inhibited the facilitating effect of PGE$_2$ and preserved haemostasis

DG-041 potently inhibits EP$_3$ on human platelets (see Supplementary material online, Figure S4). In healthy volunteers ingesting DG-041 for 7 days, the twice-daily 100 mg dose abrogated the ex vivo platelet aggregation induced by sub-threshold collagen in the presence of 10$^{-6}$ M
Figure 4  
PGE$_2$ was produced by human plaques and altered human platelet function in whole blood. (A) PGE$_2$ produced by stenotic (‘culprit’: C, $n = 10$) or less stenotic (‘non-culprit’: NC, $n = 10$) human plaque fragments vs. atherosclerosis-free arterial samples ($n = 11$) incubated in culture media for 24 h. (B) Top left, a typical trace of platelet aggregation in whole human blood in response to sub-maximal activation induced by U46619 (200 nmol/L) after adding PGE$_2$ ($10^{-7}$ mol/L) vs. saline. Below is another example in which the facilitating effect of PGE$_2$ was minor at 5 min, but more important at 8 min. Right: data from the nine volunteers are reported in ohms (top) measured at 5 min or at maximal aggregation, and below as AUC at 5 or 8 min. Each point is the mean of three replicates. (C) Dephosphorylation of pVASP (i.e. low pVASP ratio) by ADP or sulprostone in patients with peripheral artery occlusive disease (PAOD) medicated with clopidogrel ($n = 5$) or aspirin ($n = 5$) when compared with disease-free platelet donors (controls, $n = 6$). Blocking P$_2$Y$_12$, with clopidogrel prevented pVASP dephosphorylation by ADP, not by the EP$_3$ agonist sulprostone. DG-041 prevented pVASP dephosphorylation by sulprostone in all groups; *$p < 0.05$, **$p < 0.005$, #$p < 0.001$. 

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The effect of EP3 blockade on the scratching model confirms that the PGE2 amount produced by plaque inflammation is sufficient to sensitize platelets. Moreover, it was not known whether it could re-sensitize platelets that have been blocked by conventional anti-platelet drugs. From the total amount of PGE2 detected in Apoe−/− aorta,9 the average number of plaques (10) in our 50- to 60-week-old mice and murine plaque volumes (0.1–0.001 μL as measured by micro-CT36,37), we calculated that the PGE2 concentration in murine plaques varies from 1 to >400 μmol/L. Here, 1 μmol/L of PGE2 was sufficient to re-sensitize murine clopidogrel-blocked platelets, consistent with the decrease of pVASP observed in human clopidogrel-blocked platelets in response to suprostone. Thus, plaque-produced PGE2 can reduce the efficacy of conventional anti-platelet treatments, and platelets from clopidogrel-treated patients which appear insensitive to ADP in ex vivo clinical aggregation tests may actually become reactive on contact with ruptured atherosclerotic plaques, which release PGE2. These sensitizing and re-sensitizing effects of PGE2 pinpoint the EP3 receptor as an attractive target to control thrombosis.

Two previous studies showed opposed results on bleeding times in Ep3−/− mice.11,12 Here, blocking EP3 did not significantly alter bleeding times after tail transection or vein incision, neither did it alter blood loss after tail transection. Two other approaches (peritoneal RBC counting and brain slice imaging) led to the same conclusion. Based on these five different approaches in the three organs, we concluded that the PGE2/EP3 pathway does not interfere with murine haemostasis. This is in agreement with our failure to detect any significant production of PGE2 at the injury site; it is likely that inflammation is too slow a process to play any significant role in urgent haemostasis.

Thus, targeting EP3 should dissociate thrombosis from haemostasis in humans, provided that the mechanisms at play are similar.

The PGE2 content of human lipid-rich plaques excised from atherosclerotic arteries was previously found too low to alter blood platelet function.22 We similarly obtained negative results when we measured PGE2 in isolated plaques. Conversely, we detected PGE2 after mashing aortas immediately after harvest or while unfreezing,9 which suggests that excising plaques from surrounding tissues degrade PGE2. Here, human plaques quickly immersed in culture media produced PGE2, demonstrating that they can produce it. The same authors22 reported that PGE2 did not alter human platelet aggregation in blood, as shown by the 5-min area under the curve (AUC).21 We reproduced similar data (P = 0.063); however, maximal aggregations at 5- and 8-min (ohms) and the 8-min AUC were significantly increased. This late response in some individuals may be due to initial higher cAMP platelet contents. Thus, PGE2 is produced by human plaques, facilitates platelet aggregation in whole blood, and therefore can impact atherothrombosis.

Consistent with the lack of any impact on murine haemostasis and in agreement with another recent study,18 DG-041 up to 1600 mg/day (eight times the dose that inhibits the facilitating effect of PGE2 on platelets) did not alter the human bleeding time. Hence, blocking EP3 opens the possibility to reduce thrombosis without altering haemostasis in humans.

Apart from aspirin, the efficacy of which is limited by bleeding, NSAIDs, or COX-2 blockers increased cardiovascular events in clinical trials,39–41 although they inhibit PGE2 production. Actually, these drugs inhibit the entire prostaglandin pathway, unfavourably impacting the balance PGI2/TXA2 and increasing blood pressure.42 Conversely, blocking specifically the PGE2/EP3 pathway should preserve other prostaglandin functions and avoid vasocstriction.43,44 Blocking the production of PGE2 will also inhibit its multiple physiological functions. Hence, blocking

4. Discussion

Atherothrombosis remains a clinical problem, partly because intensifying anti-thrombotic treatment exacerbates the risk of bleeding.15 The PGE2/EP3 pathway is of interest because PGE2, which facilitates the aggregation of activated platelets whatever the activator,13 is produced in much larger quantities by atherosclerotic plaques than by healthy vascular walls.7

Since we have previously demonstrated that PGE2 is functional in plaques and activates its EP3 receptor on platelets in the vicinity,9 it was not surprising that DG-041 reduced atherothrombosis. This effect results not only from the EP3 blockade, but also from PGE2 activation of EP2 and EP4, which synergizes with the EP3 blockade to increase the intraplatelet level of cAMP and to inhibit platelet function.13–17

Figure 5 DG-041 inhibited human platelet aggregation, but not human cutaneous haemostasis. Bleeding times (top, n = 29, 6, 8, 8, and 8, respectively) and aggregation tests (below, n = 17, 5, 5, 5, and 8, respectively) were measured in 30 volunteers given increasing doses of DG-041 twice a day for 7 days. Platelets were stimulated with 0.25 μg/mL of collagen and 10−6 mol/L of PGE2. Pre-dose: basal value, plac: placebo. *P < 0.05.

PGE2 (4.67 ± 0.89%, n = 5 vs. 73.94 ± 1.60%, n = 17 measured before treatment, Figure 5—bottom), Although platelet response to collagen plus PGE2 was inhibited by DG-041, bleeding times were not altered, even by a twice-daily 800 mg dose (4.55 ± 0.64 min, n = 8 vs. 3.90 ± 0.20 min, n = 30 measured before treatment, Figure 5—top). Thus, inhibiting the PGE2/EP3 pathway with DG-041 reduced the aggregation of human platelets, but did not alter cutaneous haemostasis in patients.
specifically the EP3 receptor is an attractive strategy. The EP3 receptor has been detected at significant levels in the kidneys, pancreas, adipocytes, and uterus, but EP3-deficient mice did not display renal, metabolic, or fertility disorders. In human volunteers (Clinical Study Reports for trials DG-041-CV-007, DG-041-CV-008), neither renal function nor glucose levels were altered by DG-041 at doses up to 1600 mg daily for 28 days.

Compared with other strategies developed to preserve haemostatic function, targeting the PGE2/EP3 pathway allows for the restriction of the anti-thrombotic effect to the plaque site, since plaques produce PGE2 as opposed to the non-inflamed bleeding site. Since PGE2 also reduces the protection conferred by clopidogrel, blocking the EP3 receptor increases platelet inhibition at the ruptured site, where anti-thrombosis is crucial.

To conclude, PGE2 is produced by human atherosclerotic plaques and facilitated platelet function in human blood. Blocking EP3 in mice or human volunteers inhibited platelet function while preserving haemostatic function. Thus, haemostasis and thrombosis differ by the selective involvement of the PGE2/EP3 pathway, and therefore targeting the EP3 receptor should increase the efficiency of conventional anti-platelet drugs without increasing the bleeding risk in myocardial infarction or strokes.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

Conflict of interests: J.E.F. was a consultant at deCODE Genetics for 1 year (2008). K.S. is deCODE Genetics’ CEO and M.E.G. is a former employee of deCODE Genetics.

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