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### **Prostaglandin E<sub>2</sub> acts via the EP<sub>4</sub> receptor to inhibit platelet aggregation**

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#### **Background**

Platelets play a central role in haemostasis. Blood vessel injury leads to platelet aggregation and also invokes an inflammatory response leading to the formation of prostanoids like prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and prostacyclin (PGI<sub>2</sub>). It is known that low concentrations of PGE<sub>2</sub> enhance and high concentrations inhibit platelet aggregation. PGE<sub>2</sub> mediates its effect through four receptors: EP<sub>1</sub> (G $\alpha_q$  signalling), EP<sub>3</sub> (three isoforms present; signals via G<sub>i</sub>, G<sub>s</sub> or G<sub>q</sub> based on cell type), EP<sub>2</sub> and EP<sub>4</sub> (G<sub>s</sub> signalling). PGI<sub>2</sub> is known to inhibit platelet aggregation through its IP receptor (G<sub>s</sub> signalling). The role of EP<sub>3</sub> in exacerbating platelet aggregation has been well described. But the role of EP<sub>4</sub> which acts via the same G protein coupling like IP has not been explored in detail. The aim of this study was to investigate the role of EP<sub>4</sub> in platelet aggregation.

#### **Methods**

Platelet aggregation assays were performed *ex vivo* using a platelet aggregation analyser (Aggregometer II). Blood from healthy human donors was used to obtain platelet-rich plasma. Aggregation was induced using ADP or collagen. Different agonists and antagonists were added to investigate their effects on platelet aggregation. Ca<sup>2+</sup> flux changes caused by addition of agonists were also examined using a fluorescent Ca<sup>2+</sup> dye (Fluo-3 AM) by flow cytometry.

#### **Results**

As expected, PGE<sub>2</sub> (up to 300 nM) and an EP<sub>3</sub> agonist (sulprostone) enhanced platelet aggregation, whereas an EP<sub>2</sub>-selective agonist (butaprost) seemed to have no effect on platelet aggregation. On the contrary, an EP<sub>4</sub> agonist (ONO AE1-329) inhibited platelet aggregation in a concentration-dependent manner, and this effect could be reversed by using EP<sub>4</sub> antagonists (ONO AE3-208 and GW627368x) but not an IP or a DP antagonist. Inhibition of protein kinase C prevented the inhibitory effect of the EP<sub>4</sub> agonist, while inhibition of adenylate cyclase had no effect. The EP<sub>4</sub> agonist ONO AE1-329 also attenuated Ca<sup>2+</sup> flux in platelets that had been stimulated with ADP.

#### **Conclusions**

These results are suggestive of an exclusive EP<sub>4</sub> effect on inhibition of platelet aggregation and EP<sub>4</sub> could be a potential target of antithrombotic therapy.