Platelet glycoprotein IIb/IIIa blockade with tirofiban: effect on aggregation caused by P256, an antibody to human IIb/IIIa receptors

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Aims P256 is a divalent antibody which aggregates human platelets by interaction with glycoprotein (GP) IIb/IIIa receptors. We investigated the effect of tirofiban, an antagonist of the GP IIb/IIIa receptor, on P256-mediated platelet aggregation.

Methods Responses to agonists were measured turbidometrically at 37°C in stirred citrated platelet-rich plasma from venous blood samples from healthy human volunteers. Inhibitory effects were determined by comparison with aggregation to the same concentration of agonist in a vehicle treated sample.

Results Tirofiban inhibited a near maximally effective dose of P256 (10^{-7} mol l⁻¹) with an IC₅₀ of 9.3×10^{-8} mol l⁻¹. Tirofiban (10^{-7} mol l⁻¹) inhibited responses to arachidonic acid, U46619 and P256 similarly, whereas aspirin (1.1×10^{-4} mol l⁻¹) inhibited arachidonic acid more effectively than P256 ($P < 0.007$ by ANOVA).

Conclusions Tirofiban potently and selectively inhibits P256-stimulated aggregation of human platelets.

Keywords: arachidonic acid, aspirin, glycoprotein receptors, platelets, tirofiban

Introduction

Glycoprotein (GP) IIb/IIIa receptors play a key role in platelet aggregation [1]. Distinct signal transduction pathways for different agonists converge on activation of GP IIb/IIIa receptors. P256 is a divalent mouse monoclonal antibody which recognizes an epitope on the IIb component of primate platelets [2]. It induces fibrinogen binding to human platelets, increases cytoplasmic calcium and causes aggregation [3].

There is interest in the therapeutic potential of drugs that inhibit GP IIb/IIIa. Abciximab, an antibody directed against GP IIb/IIIa receptors, reduced thrombotic complications following angioplasty in high risk patients when compared with a combination of aspirin and heparin, but at the expense of increased bleeding [4]. Tirofiban [5] is a low molecular weight (M_r 495), nonpeptide tyrosine analogue that is a highly selective inhibitor of fibrinogen binding to GP IIb/IIIa. We investigated the effect of tirofiban on P256-mediated platelet aggregation.

Methods

Healthy volunteers of either sex were recruited from Medical School staff. None was taking medication.

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Citrated platelet-rich (PRP) and platelet-poor plasma (PPP) were prepared by differential centrifugation. Portions (500 µl) were transferred to stirred cuvettes in a two-channel Payton 600B aggregometer. The difference in light transmission between PRP and PPP was taken as 100% aggregation. PRP was equilibrated at 37°C for 1 min before addition of antagonist (or vehicle control) and for a further minute before addition of agonist. Tirofiban and U46619 (15S-hydroxy-11 α , 9 α -epoxymethanoprostano-5Z, 13E-dienoic acid) were dissolved in saline (0.9% NaCl). Aspirin was dissolved in tris buffer (1 mol l⁻¹, pH 8.4). Arachidonic acid was dissolved in ethanol. Equal volumes of solvents were added to control PRP. P256 was a generous gift from Cynthia Dixon (Imperial Cancer Research Foundation, Lincoln's Inn Fields, London, UK); tirofiban was a gift from Merck, Sharp & Dohme (Hoddesdon, Herts, UK); abciximab (ReoProTM) was obtained from Lilly, UK; other drugs and reagents were from Sigma (Poole, Dorset, UK). Aggregation was measured 180 s after addition of agonist. The effect of antagonist was expressed as percentage inhibition of aggregation in the control sample.

Statistical analysis

Values are summarised as means $\pm$ s.e. mean. Dose-response curves were analysed by PrismTM (version 2.01). Effects of antagonists were analysed using a summary

score to describe effects of agonists in the presence of antagonist or of vehicle [6] followed by analysis of variance. A P value of less than 0.05 was considered to indicate statistical significance.

Results

P256 caused dose-related platelet aggregation ($EC_{50} = 1.1 \pm 0.1 \times 10^{-8} \text{ mol l}^{-1}$; $E_{\max} = 73.4 \pm 4.1\%$, Figure 1a). A near-maximally effective concentration of P256 of $10^{-7} \text{ mol l}^{-1}$ was used to measure the effect of tirofiban on P256-induced aggregation. Tirofiban inhibited P256-induced platelet aggregation with a steep dose response relationship, $IC_{50} = 9.3 \pm 1.1 \times 10^{-8} \text{ mol l}^{-1}$ and $E_{\max} = 89.8 \pm 3.9\%$ inhibition (Figure 1b).

Effects of tirofiban ($10^{-7} \text{ mol l}^{-1}$) on P256-induced aggregation were further compared in separate experiments with its effects on arachidonic acid and U46619-induced aggregation. Tirofiban had similar inhibitory effects on P256 and arachidonic acid, whereas aspirin ($1.1 \times 10^{-4} \text{ mol l}^{-1}$) inhibited arachidonic acid

more than P256 (Figure 2, $n=8$, $P<0.007$). Aspirin inhibited the highest dose of P256 only by $21.2 \pm 7.7\%$. In separate experiments, tirofiban ($10^{-7} \text{ mol l}^{-1}$) similarly ($P>0.8$) and profoundly ($>80\%$) inhibited P256 and U46619.

Another antagonist of the IIb/IIIa receptor, abciximab ($4.2 \times 10^{-7} \text{ mol l}^{-1}$) inhibited the effect of P256 ($10^{-7} \text{ mol l}^{-1}$) by $68.6 \pm 2.3\%$.

Discussion

Antiplatelet drugs have an important place in the treatment and prevention of vascular disease. Aspirin is the main antiplatelet drug in clinical use. It inhibits arachidonic acid initiated/thromboxane A_2 mediated aggregation [7]. However, full aggregation can occur despite the presence of aspirin in response to sufficient stimulation by other agonists such as collagen, thrombin and serotonin. Antiplatelet drugs with a wider range of inhibitory effects than COX inhibitors could have greater therapeutic benefit than aspirin. Inhibitors of GP IIb/IIIa receptors are particularly attractive candidates in this

Figure 1 Concentration of P256 vs response (% aggregation) of human platelet rich plasma at 37°C (a; $n=8$). b) shows the concentration of tirofiban vs % inhibition of aggregation to P256 ($10^{-7} \text{ mol l}^{-1}$; $n=8$).

Figure 2 Concentration effect curves ($n=8$) of arachidonic acid (a) and P256 (b) with tirofiban $10^{-7} \text{ mol l}^{-1}$ (■), aspirin $1.1 \times 10^{-4} \text{ mol l}^{-1}$ (▲), and vehicle alone (●).

regard, because of the key role of these receptors in the final common pathway to platelet aggregation. Positive effects of abciximab [4] support this possibility. Disadvantages of antibodies as therapeutic agents have led to the development of low molecular weight inhibitors of GP IIb/IIIa receptors such as tirofiban. Clinical studies have shown improved outcomes with tirofiban, particularly when used in combination with heparin [8]. These benefits have been seen using weight-adjusted infusion rates, rather than doses based on any individualized measure of platelet aggregation, which are not currently routinely available and for which a therapeutic range has yet to be established.

Evidence that P256 is a GPIIb/IIIa agonist is indirect. It recognizes an epitope on human GP IIb [2], and its effect on aggregation is antagonized by a monovalent Fab fragment of the antibody which binds to a single saturable binding site on human gel-filtered platelets [3]. P256 does not merely agglutinate platelets by binding bivalently to receptors on adjacent platelets, but causes active aggregation associated with a rise in cytoplasmic Ca^{2+} and is blocked by prostacyclin [3]. This is supported by the present observation that the response to P256 is antagonized by abciximab. The main finding of the present study is that tirofiban inhibits platelet aggregation responses to P256, as well as to arachidonic acid and to U46619. This contrasts with aspirin, which is relatively selective for responses to arachidonic acid. Aspirin does have a small inhibitory effect on responses to P256, consistent with previous observations with indomethacin [3], presumably because P256 secondarily activates phospholipase, liberates arachidonic acid and hence augments aggregation through formation of thromboxane A_2 . The much more potent inhibitory effect of tirofiban on responses to P256 suggests that P256 may be of value in future experiments to investigate effects of GP IIb/IIIa

receptor antagonists *ex vivo*, including investigations where patients are also receiving aspirin or other platelet antagonists.

We conclude that P256 provides a tool for measuring GP IIb/IIIa receptor antagonism. This may prove useful in selecting doses of agents for clinical assessment.

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