Role of phosphatidylinositol 3-kinase in human platelet aggregation

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Platelet aggregation plays an important role in haemostasis and vascular disorders. This mainly takes place by the action of endogenous agonists like ADP, PAF, epinephrine, 5-HT and arachidonic acid (AA). These agonists except AA interact with G-protein coupled receptors [1]. In platelets, IP3 causes release of calcium from internal stores and DAG activates protein kinase C (PKC). PKC acts in synergy with Ca++ mobilization for the activation of platelets. Recent studies have shown that phosphatidylinositol 3-kinase (PI 3-kinase) and myosin light chain kinase (MLCK) play important role in platelet aggregation [2,3]. Wortmannin, a compound derived from *Pencillium wortmannin* has been shown to be a selective and potent inhibitor of PI 3-kinase at very low doses (1-10 nM) and it also inhibits MLCK at higher doses (> 100 nM) [4]. We have recently shown that wortmannin in very low doses inhibits platelet aggregation induced by synergistic action of epinephrine and 5-HT [5]. In continuation to our previous work, here we studied the effect of PI 3-kinase by using wortmannin in human platelet aggregation in response to various platelet agonists.

Blood samples from normal human volunteers reported to be free of medication for one week were mixed with 3.8% (w/v) sodium citrate (9:1) and centrifuged at 260 g for 15 min at 20°C to obtain platelet-rich plasma (PRP). Aggregation was monitored using a Dual-channel Lumi aggregometer with 0.45 ml aliquots of PRP for 5 minutes [4]. The final volume was made up to 0.5 ml with sodium citrate (0.9%, w/v) or the test drugs and incubated for 1 min before challenge with the aggregating agent. The release of 5-HT was measured as described [6]. To examine any changes produced in the expression and relocation of PI 3-kinase from the cytosol to cytoskeleton, we did Western blots (8% SDS-PAGE) using antisera raised against PI 3-kinase in rabbits [7].

The platelet agonists (epinephrine, ADP, AA, collagen and PAF) increased the platelet aggregation in a dose dependent manner. The maximal effect produced by these agonists occurred at concentrations as following; epinephrine (20 μM), ADP (4.2 μM), collagen (638 nM), AA (1.7 mM) and PAF (0.8 μM). Treatment of platelets in PRP with wortmannin alone up to doses of 1 μM had no effect on platelets, however, wortmannin inhibited the aggregation induced by maximal concentrations of these agonists with varying inhibitory concentrations (IC50, 110-520 nM) as shown in (Table 1). Our results also show that 2 and 4 μM of wortmannin caused 10 and 62 % decrease in epinephrine-induced release of 5-HT in platelets. Epinephrine alone or in combination with low dose of 5-HT caused a shift in PI 3-kinase from cytosol to cytoskeleton (40%) and this effect was blocked by wortmannin. PI 3-kinase is a cytosolic enzyme and has been reported to play a trivial role in the platelet aggregation through phosphorylation cascade.

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Table 1: Inhibitory effects of wortmannin on platelet aggregation induced by various platelet agonists. The values are IC50 of wortmannin (n=4).

<table>
<thead>
<tr>
<th>Agonist (concentration)</th>
<th>IC50 μM</th>
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<tbody>
<tr>
<td>ADP (4.2 μM)</td>
<td>110 μM</td>
</tr>
<tr>
<td>Collagen (638 nM)</td>
<td>280 nM</td>
</tr>
<tr>
<td>PAF (0.8 nM)</td>
<td>520 nM</td>
</tr>
<tr>
<td>Epinephrine (20 μM)</td>
<td>2.5 μM</td>
</tr>
<tr>
<td>Epinephrine + 5-HT (5 μM)</td>
<td>20 nM</td>
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In conclusion, both PI 3-kinase and MLCK seem to play vital role in the platelet aggregation through phosphorylation cascade.