**Agonist-induced protein disulfide isomerase activity in platelets: different effects of different agonists**

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**Introduction**

Protein disulfide isomerase (PDI) resides mainly in the endoplasmic reticulum and assists in protein folding. PDI is a member of the thiol isomerase family that includes at least 21 family members in mammals. These enzymes can mediate reduction, oxidation and isomerization (exchange) of disulfide bonds in proteins (1). PDI is also displayed on the surfaces of various cells including endothelial cells and platelets. PDI is released from platelets following their activation and facilitates platelet aggregation and thrombus formation in mice thrombosis models (2,3).

**Aims**

To investigate the effect of different agonists on PDI activity in human platelets and explore whether this effect correlates with platelet activation.

**Methods**

Washed platelets were prepared from healthy controls’ blood samples. Enzymatic reductase activity in platelets that were either activated or not by various agonists was assayed by measuring the kinetics of eosin 5-isothiocyanate-coupled glutathione disulfide (Di-E-GSSG) reduction. The contribution of PDI to reductase activity was assessed by adding a specific PDI inhibitor, quercetin-3-rutinoside (rutin). Platelet activation by the various agonists was measured by flow cytometry (FACS) using antibodies against the platelet activation markers p-selectin (CD62P) and active αIIBβ3 (PAC-1).

**Results**

**Disulfide reductase activity was increased following platelets activation by some agonists but not by all agonists**

Disulfide reductase activity was measured over 120 minutes in agonist-induced activated platelets compared to non-activated platelets (None). Disulfide reductase activity was increased following platelets activation with ionophore (10 µM), Convolxin (20 ng/ml), TRAP (200 µM), U46619 (7.5 µM) and Human thrombin (H-Thrombin, 0.5 U/ml). Disulfide reductase activity was not increased following platelets activation with Collagen (10 μg/ml) or with ADP in different concentrations (2, 5 and 10 μM). ADP (10 μM) also did not further increase reductase activity induced with TRAP.

Disulfide reductase activity in different individuals was significantly increased following platelets activation by TRAP, U46619 and Human thrombin.

Disulfide reductase activity was measured in platelets taken from 6 or 7 healthy controls. Following platelets activation with TRAP, U46619 and H-Thrombin, both the end point values of reductase activity at 120 min (left) and the initial rates of reductase activity assessed by the slopes of the first 20 minutes of each curve (right) were significantly increased (**,** P<0.01; ***,** P<0.001).

**PDI is responsible for the increased reductase activity upon platelets activation**

Addition of rutin decreased reductase activity induced by U46619, TRAP or H-Thrombin and also of resting platelets (none). This is shown by the statistically significant reduction of both the end point (left) and the initial rate (right) of Di-E-GSSG cleavage following rutin addition. Bars represent mean±SE of 4-10 experiments (*, P<0.05 ; **, P<0.01; ***, P<0.001). The reduction in reductase activity was nearly to the same extent of resting platelets indicating that the increased reductase activity is derived from newly released PDI from agonist-induced activated platelets.

Platelets activation markers were profoundly increased by all agonists except for Collagen and ADP.

All agonists induced platelet activation as displayed by a statistically significant increased p-selectin expression and αIIBβ3 activation (PAC-1 binding) by FACS analysis (bars represent the mean±SE of at least 3 experiments). However, while TRAP, U46619, H-Thrombin, Convolxin and Ionophore caused a profound increase in platelets activation markers, ADP and Collagen displayed only slight increase of these markers. ADP and Collagen were also the only agonists that did not increase platelets reductase activity implying that the rise in platelets reductase activity requires strong platelet activation.

**Conclusions**

- Resting human platelets harbor active PDI on their surfaces. Some agonists that cause strong platelets activation induces the release of additional active PDI.
- Agonists that caused lesser activation of platelets did not induce PDI release from platelets, indicating that PDI release requires strong platelets activation.

**References**

1. Wilkinson B and Gilbert HF. 2004; Biochim Biophys Acta 1699: 35-44.