Cell surface protein disulphide isomerase expression on platelets and vascular wall cells


**Introduction** Protein disulphide isomerase located at the cell surface of platelets (csPDI) regulates aggregation and adhesion via activation of integrins II3 and 21, and also mediates NO-related signalling. In addition, it may contribute to activation of tissue factor on monocytes and cells of the vessel wall. In the past, PDI activity measurement has involved cumbersome methods. The aim of this study was to develop techniques for rapid and sensitive detection of functionally active csPDI.

**Methods** Washed human platelets were obtained from peripheral blood by centrifugation and separation on Sepharose 2B. Reductase activity of csPDI was monitored using the synthetic pseudosubstrate dieosin glutathione disulfide (Di-E-GSSG), generated by incubation of GSSG with a 10-fold molar excess of eosin isothiocyanate (as described by Raturi A. et al.). Platelets (0.2 - 6 x 10^7 per well) were incubated in microplate wells with 150 nM Di-E-GSSG and fluorescence (optimised as excitation at 510 nM and emission at 550 nM) was monitored over 120 minutes in a plate reader (Molecular Devices). In further experiments, platelets were incubated with the PDI inhibitor phenylarsine oxide (PAO) (12.5 - 100 μM) and csPDI activity measured.

Human megakaryocyte cell line (MEG-01) was cultured in RPMI-1640 with 20% FBS, 100 units/ml of penicillin, 100 μg/ml streptomycin and 2 mmol/L L-glutamine. Human coronary artery endothelial (HCAEC) and smooth muscle cells (HCASMC) were purchased from PromoCell and cultured using the company-provided medium. Expression of csPDI on these cells was analyzed using flow cytometry using PE-labelled anti-PDI antibody (1D3). Results were expressed as mean fluorescence density, relative to isotype control.

**Results** Platelet csPDI showed cell number-dependent reductase activity that was inhibited in a concentration-dependent manner by PAO, with rate of fluorescence falling from 0.148 RFU/min in untreated control cells to 0.056 RFU/min in cells treated with 100 μM PAO (62% inhibition compared with untreated cells). Flow cytometry analysis revealed the presence of csPDI on platelets and all three types of cultured cells. Compared with platelets, the expression of PDI was significantly higher on MEG-01 (p<0.01) and HCASMC (p<0.05).

**Conclusions** A csPDI activity assay in 96 well format, suitable for high throughput studies, has been established, as has a flow cytometric technique to detect immunoreactive csPDI on platelets and other cell types. Increased levels of csPDI on MEG-01 megakaryocytes, relative to platelets, may reflect changes occurring during the platelet maturation process. Differences in csPDI expression between platelets and vascular wall cells may influence csPDI-dependent processes in platelet regulation and progression of atherosclerotic disease. csPDI is emerging as a key regulator of haemostasis, and understanding the regulation of its expression and activity may offer important new insights and therapeutic possibilities. The development of sensitive and convenient means of investigating csPDI will be crucial to address these issues.


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