Role of Cell Surface Protein Disulphide Isomerase in the Platelet-Selective Action of S-Nitrosoglutathione

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Introduction

Administration of S-nitrosoglutathione (GSNO) to patients is reported to inhibit platelets at doses that produce no vasodilatation, suggesting a platelet-selective effect that might be useful in antithrombotic therapy, however the mechanism involved remains unknown. We previously showed that cell surface protein disulphide isomerase (csPDI) was required for rapid NO delivery into human platelets from NO donors. We have now explored expression and activity of csPDI on platelets and cells of the vascular wall, and their dependence on PDI for NO delivery from GSNO, to further define the platelet-selective properties of GSNO.

Methods

Washed human platelets were obtained by centrifugation and separation on Sepharose 2B. Human coronary artery endothelial (HCAEC) and smooth muscle cells (HCASMC) were purchased from PromoCell and cultured up to 3–5 passages. Expression of csPDI was analyzed using flow cytometry. csPDI reductase activity was monitored using synthetic pseudosubstrate dyesolin glutathione disulfide (Di-E-GSSG) in a microplate fluorescence assay. To determine the effect of cell activation, the three cell types were incubated with phorbol myristate acetate (PMA) or ionomycin (1-100 μM), prior to measurement of csPDI activity.

Intracellular delivery of NO from GSNO (0.1 – 100 μM) was measured fluorometrically using DAF-FM. In further experiments, csPDI on platelets, HCAEC and HCASMC was inhibited with 100 μM of phenylarsine oxide (PAO), prior to addition of 1 mM GSNO.

Results

Flow cytometry revealed csPDI on all three cell types, but percentage positivity was higher on platelets than on HCAEC or HCASMC (p < 0.01). Compared with both HCAEC and HCASMC, csPDI reductase activity was also higher on platelets (p < 0.01). Cellular activation (with either PMA or ionomycin) increased csPDI activity on both platelets and smooth muscle cells (p < 0.01 compared with untreated controls), but not on endothelium. Intracellular NO delivery from GSNO was greater in platelets, compared with HCAEC and HCASMC (P < 0.05), and PAO caused a greater degree of inhibition of NO delivery in platelets than in vascular cells (P < 0.05).

Conclusions

Platelets show higher activity of csPDI than endothelial or vascular smooth muscle cells, and this coincides with increased NO delivery from GSNO. Higher activity of csPDI in platelets, compared with cells of vascular wall, may explain the platelet-selective actions of GSNO.

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