Expression of matrix meyalloproteinase-9 in human platelets: regulation of platelet activation in in vitro and in vivo studies

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摘要

Abstract

1. The aim of this study was to identify the presence of matrix metalloproteinase-9 (MMP-9) in human platelets and systematically examine its inhibitory mechanisms of platelet activation. 2. In this study, we report on an efficient method for the quantitative analysis of pro-MMP-9 in human platelets using capillary zone electrophoresis (CZE). To elucidate subcellular localization of MMP-9 in human platelets, we investigated intraplatelet MMP-9 by immunogold labeling and visualized it using electron microscopy. In an in vivo thrombotic study, platelet thrombus formation was induced by irradiation of mesenteric venules with filtered light in mice pretreated with fluorescein sodium. 3. MMP-9-gold labeling was observed on the plasma membrane, alpha-granules, open canalicular system, and within the cytoplasma both in resting and activated platelets. Furthermore, activated MMP-9 concentration-dependently (15-90 ng ml(-1)) inhibited platelet aggregation stimulated by agonists. Activated MMP-9 (21 and 90 ng ml(-1)) inhibited phosphoinositide breakdown, intracellular Ca(2+) mobilization, and thromboxane A(2) formation in human platelets stimulated by collagen (1 microg ml(-1)). In addition, activated MMP-9 (21 and 90 ng ml(-1)) significantly increased the formation of nitric oxide/cyclic GMP. 4. Rapid phosphorylation of a platelet protein of Mr 47,000 (P47), a marker of protein kinase C activation, was triggered by phorbol-12, 13-dibutyrate (PDBu) (60 nm). This phosphorylation was markedly inhibited by activated MMP-9 (21 and 90 ng ml(-1)). Activated MMP-9 (1 microg g(-1)) significantly prolonged the latency period of inducing platelet plug formation in mesenteric venules. 5. These results indicate that the antiplatelet activity of activated MMP-9 may be involved in the following pathways. (1)

Activated MMP-9 may inhibit the activation of phospholipase C, followed by inhibition of phosphoinositide breakdown, protein kinase C activation, and thromboxane A(2) formation, thereby leading to inhibition of intracellular Ca(2+) mobilization. (2) Activated MMP-9 also activated the formation of nitric oxide/cyclic GMP, resulting in inhibition of platelet aggregation. These results strongly indicate that MMP-9 is a potent inhibitor of aggregation. It may play an important role as a negative feedback regulator during platelet activation