# Inhibitory mechanisms of metallothionein on platelet aggregation in in vitro and platelet plug formation in in vivo experiments.

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## 摘要

## Abstract

Metallothionein (MT) is a low-molecular-weight, cysteine-rich protein that contains heavy metals such as cadmium and zinc. The biological function of MT in platelets is not yet understood. Therefore, the aim of this study was to systematically examine the inhibitory mechanisms of metallothionein in platelet aggregation. In this study, metallothionein concentration-dependently (1-8 μM) inhibited platelet aggregation in human platelets stimulated by agonists. Metallothionein (4 and 8 μM) inhibited phosphoinositide breakdown in [3H]-inositol-labeled platelets, intracellular Ca+2 mobilization in Fura-2 AM-loaded platelets, and thromboxane A2 formation stimulated by collagen. In addition, metallothionein (4 and 8 μM) significantly increased the formation of cyclic GMP but not cyclic AMP in human platelets. Rapid phosphorylation of a protein of Mr 47,000 (P47), a marker of protein kinase C activation, was triggered by PDBu (100 nM). This phosphorylation was markedly inhibited by metallothionein (4 and 8 μM) in phosphorus-32-labeled platelets. In an in vivo thrombotic study, platelet thrombus formation was induced by irradiation of mesenteric venules in mice pretreated with fluorescein sodium. Metallothionein (6 μg/g) significantly prolonged the latency period for inducing platelet plug formation in mesenteric venules. These results indicate that the antiplatelet activity of metallothionein may involve the following pathways: (1) metallothionein may inhibit the activation of phospholipase C, followed by inhibition of phosphoinositide breakdown and thromboxane A2 formation, thereby leading to inhibition of intracellular Ca+2 mobilization; (ii) Metallothionein also activated the formation of cyclic GMP in human platelets, resulting in inhibition of platelet aggregation. The results

strongly indicate that metallothionein provides protection against thromboembolism.