

ubiquitinated proteins in the platelets contained ubiquitinated COX-1, whose levels were correlated with the expressions of ubiquitinated proteins. Inhibition of CXCR4 by AMD3100 caused a dose-related decline in ubiquitinated COX-1, which was correlated positively with platelet activity.

Conclusions The data suggested that extracellular Ub dose dependently, via CXCR4 pathway, facilitated its internalisation into the platelet to enhance the ubiquitination of COX-1, which contributed to the increase in platelet activity.

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EXTRACELLULAR UBIQUITIN VIA CXC CHEMOKINE RECEPTOR 4 ENHANCES PLATELET ACTIVITY BY UBIQUITINATION OF CYCLOOXYGENASE-1

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Objectives To investigate the mechanism of extracellular ubiquitin (Ub) influence on the platelet functions.

Methods The arachidonic acid (AA)-preincubated healthy platelets were treated with different concentrations of extracellular Ub (50, 100, 500 and 1000 ng/ml), or AMD3100 (50, 100, 500 and 1000 ng/ml) (antagonist of CXCR4) prior to extracellular Ub (1000 ng/ml). Platelet functions were determined by light-transmission platelet aggregometry (LTA) or thrombelastography (TEG) platelet mapping. The protein expressions of CXCR4 and ubiquitinated proteins, including ubiquitinated COX-1, were detected by western blot or Immunoprecipitation (IP).

Results LTA or TEG showed that the activity of platelet exposed to extracellular Ub at 100, 500 and 1000 ng/ml was significantly increased compared with that at 50ng/ml. The increased activity was positively correlated with the levels of ubiquitinated proteins in the platelets. However, AMD3100 dose dependently counteracted the effect of Ub, and the platelet aggregation decreased accordingly. Further, CXCR4 could be activated by extracellular Ub or inhibited by AMD3100 dose dependently, and the level of CXCR4 was correlated with the ubiquitinated proteins, including ubiquitinated COX-1, in the platelets. IP experiments showed that the