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Poster presentation

NO inhibits platelet apoptosis by cGMP-dependent and-independent pathways

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Background

Platelets are specialized anucleate cells that play key roles in hemostasis through their ability to rapidly adhere to subendothelial matrix proteins and endothelial cells (platelet adhesion) and to other activated platelets (platelet aggregation). Platelet activation is an irreversible process resulting ultimately in platelet apoptosis and death. The NO-cGMP-PKG pathway plays an essential role in platelet inhibition, however the role of this pathway in regulation of platelet apoptosis has not been investigated in detail so far. Surface exposure of negatively charged phospholipid phosphatidylserine (Annexin V-binding) and loss of mitochondrial membrane potential ($\Delta\Psi_m$) of activated platelets were used as markers of platelet apoptosis induced by thrombin (up to 0.5 U/ml), convulxin (Cvx 5 ng/ml) or by a mixture of thrombin/convulxin (Thr/Cvx 0.005 U/ml/5 ng/ml).

Results

Thrombin alone, even at high concentrations, had no effect on Annexin V-binding, Cvx alone (190 ± 12%), and combination of Thr/Cvx (640 ± 60%) significantly increased Annexin V binding (mean fluorescence) after 2 min of stimulation. An NO donor (DEA-NO,1 μM) increased platelet cGMP content, induced vasodilator-stimulated phosphoprotein (VASP) phosphorylation, and significantly (48 ± 5%) inhibited Cvx-evoked Annexin V-binding in platelets. The effect of DEA-NO was significantly enhanced (up to 15%) by the PDE5 inhibitor sildenafil (5 μM) and attenuated by preincubation (5 min) with the sGC inhibitor (ODQ, 5 μM). A membrane-permeable cGMP analog (8-pCPT-cGMP, 100 μM,10 min preincubation) also induced VASP phosphorylation, however, it only slightly (17 ± 3%) inhibited Cvx-increased Annexin V-binding (all data calculated as % inhibition with Annexin V-binding in the presence of Cvx alone used 100%). When platelet apoptosis was induced with stronger stimuli (Thr/Cvx) the inhibitory effects of NO donors were less pronounced (20 ± 4%) when compared to platelets stimulated with Cvx alone. In Thr/Cvx stimulated platelets mitochondrial membrane potential ($\Delta\Psi_m$) was reduced up to 40 ± 5% compared to the control, and this effect was significantly inhibited (61 ± 12%) by DEA-NO (1 μM). However, in contrast to its effect on Annexin V-binding (see above), the DEA-NO effects on mitochondrial membrane potential ($\Delta\Psi_m$) were not attenuated by ODQ which (as positive control) completely prevented DEA-NO induced cGMP increase and VASP phosphorylation.

Conclusion

We conclude that NO inhibits platelet apoptosis by cGMP-dependent (in the case of phosphatidylserine surface exposure) and cGMP-independent (loss of mitochondrial membrane potential) mechanisms.

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