Activation of haemostatic system in cyclooxygenase-2 knockout mice (COX-2KO)

E. Tarantino°, S.S. Barbieri*, S. Gianellini*, P. Amadio°, L. Mussoni°, E. Tremoli*°

°Dipartimento di Scienze Farmacologiche e Biomolecolari (DISFeB), via Balzaretti 9, 20133 Milano, Italy
*Centro Cardiologico Monzino, IRCCS, Via Parea 4, 20138 Milano, Italy

Deep vein thrombosis (DVT) is a major cause of pulmonary thromboembolism, a leading cause of death in individuals with DVT. Several lines of evidence indicate that inhibition of cyclooxygenase-2 (COX-2) activity favour thrombotic events (Barbieri SS, et al., Circulation 2012), but the role of COX-2 in DVT remain unclear.
In this study we have assessed the effects of deletion of COX-2 on the levels or activities of haemostatic factors in relation to experimentally-induced thrombosis.
To address this issue, data obtained from COX-2 knockout (COX-2KO) mice were compared to those obtained by wild-type (WT) mice. Citrated blood was immediately analyzed by thromboelatography (ROTEM®). Levels of fibrinogen, tissue plasminogen activator (tPA), plasminogen activator inhibitor-1(PAI-1), and the activities of coagulation factors were measured in plasma. Tissue factor (TF) activity in plasma microparticles and in leukocytes was measured by one-stage plasma recalcification time assay (TF activity). Ligation of the inferior vena cava (IVC) to induce DVT in COX-2KO and WT mice was also carried out. 48 hours after thrombosis-induction mice were sacrified and thrombi were measure, dissected and analysed by histology.
Recalcification tests carried out both in whole blood or in plasma of COX-2KO mice by thromboelastography (NATEM) showed a significant increase in clot firmness and a reduction in coagulation time compared to control mice. Activation of the extrinsic or intrinsic pathways (EXTEM or INTEM) suggests that COX-2KO mice have higher levels of fibrinogen and coagulation factors than WT mice. Indeed, COX-2KO mice have augmented levels of functional fibrinogen and factor VIII and of TF, but similar levels of factors IX, XI and XII compared to WT mice. In addition, PAI-1 activity and antigen were elevated in COX-2KO compared to WT mice, whereas tPA activity was similar in the two groups.
Thrombus size was substantially greater in COX2-KO mice than in WT mice. In addition, an increased leukocyte infiltration was detected in thrombi from COX2-KO mice compared to WT mice. Remarkably, the expression of Annexin II, a fibrinolytic receptor (Brownstein C et al., 2004), was greater in leukocytes within thrombi in COX-2KO mice. Additional *in vitro* experiments were performed to further investigate the effect of COX-2 deletion on Annexin II expression in a murine macrophage line (RAW264.7). In these experiments it emerged that the inhibition of COX-2 by NS398 or by specific siRNA markedly increased the expression of Annexin II compared to control cells. In particular, in control cells Annexin II immunoreactivity was localized mainly on membrane/periphery , whereas in COX-2 silenced cells Annexin II reactivity was diffused and localized in the cytoplasm , suggesting a reduced fibrinolytic capacity of these cells.
In conclusion, the increased activation of haemostatic system observed in COX-2KO mice may partly explain the predisposition of this mouse model to thrombosis. In addition, the different expression/localization/activation of Annexin II in leukocytes from COX-2KO mice suggests an alternative mechanisms influencing the propensity to lysis of thrombi.

Barbieri SS et al. (2012) *Circulation* 126(11):1373-84
Brownstein C et al. (2004) *Blood* 103(1):317-24