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

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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 activity of haemostatic factors in relation to experimentally-induced thrombosis.
To address this issue, data obtained from COX-2 knockout (COX-2KO) were been compared to these obtained by wild-type (WT) mice. Citrated blood was immediatelly analyzed by thromboelatography (ROTEM®). Levels of fibrinogen, tissue plasminogen activator (tPA), plasminogen activator inhibitor-1(PAI-1), and coagulation factors activities were measured in plasma. Moreover, tissue factor (TF) activity of plasma microparticles and leukocytes were performed by one-stage plasma recalcification time assay. Ligation of the inferior vena cava (IVC) model to induce DVT in COX-2KO and WT mice was performed. After 48 hours mice subjected to the above thrombosis-induction models were sacrificed and thrombi were dissected, measured and histological examinations were performed.

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 bigger at 48 hours in COX2-KO mice than in WT mice. In addition, a lower leukocyte infiltration was detected in thrombi from COX2-KO mice compared to WT mice. Remarkable, the expression of annexin II, a fibrinolytic receptor (Brownstein C et al., 2004), was greater in intrathrombic leukocytes in COX-2KO mice. Additional *in vitro* experiments were performed to further investigate the effect of COX-2 deletion on Annexin II expression in murine RAW264.7 macrophage line. The inhibition of COX-2 by NS398 or by specific siRNA markedly increased the expression of Annexin II compared to the control cells. In particular, Annexin II immunoreactivity was localized mainly on membrane/periphery of control cells, whereas Annexin II reactivity was diffused and localized in the cytoplasm of COX-2 silenced cells.

In conclusion, the increased basal 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 by which thrombous from COX-2KO mice are less prone to lysis.