Effect of Cyclooxygenase-2 (COX-2) Deletion on Megakaryopoiesis and Peripheral Platelet Phenotype

S.S. Barbieri[1], G. Petrucci[2], P. Amadio[3], E. Tarantino[3], F.O. Ranelletti[2], B. Rocca[2], C. Patrono[2], E. Tremoli[1,3]

[1] Centro Cardiologico Monzino, IRCCS, Milan, [2]Catholic University School of Medicine, Rome, and [3]University of Study of Milan, Milan, Italy

Cyclooxygenase (COX)-1 or -2, thromboxane (TX) and prostaglandin (PG) synthases that together catalyse the formation of PGs (including PGI_2 and PGE_2) and TXA_2 play key roles in the maturation of different types of blood cell. While mature platelets express almost COX-1, megakaryocytes contain both COX-isoforms (Rocca B et al., PNAS 2002). *In vitro* studies showed that pharmacologic inhibition of COX-2 activity might influence megakaryocyte maturation and platelet formation (Tanaka N et al., ATVB 2004). However, the impact of COX-2 and PGs in MKs maturation and platelets function is poorly understood.

We investigated the role of COX-2 in megakaryopoiesis using a murine gene deletion model.

The phenotype and function of peripheral platelets and parent MKs isolated from COX-$2^{-/-}$(KO) mice were studied by flow cytometry, immunohistochemistry and/or functional tests.

KO peripheral platelets showed increased P-selectin and GPIIbIIIa expression, and fibrinogen binding as compared to WT platelets. Platelet TX-synthase levels were similar in WTs and KOs, while COX-1 was more expressed in KO platelets, which showed an increased TXA_2 biosynthesis. While platelet count in KO mice was slightly lower than in WTs (WT:1121±349 vs KO:1031±233 K/μl), platelet size was larger (WT:71.10±3.87 vs KO:91.27±5.48fL; $p<0.01$), with a higher percentage of young, reticulated platelets(WT:7.62±0.59 vs KO:13.27±1.01%; $p<0.01$). Histology and flow cytometry analyses showed that the MK number was significantly reduced in KO bone marrows (BM), with features of poor differentiation, such as high nucleus/cytoplasm (N/C) ratios and low expression of lineage markers (CD42d, CD49b). However, MKs were significantly increased in the KO spleens, with features of MK maturation which were absent in WT spleens. Finally, by using a pulmonary thromboembolism model to study platelet function *in vivo,* 14% of WT mice died within 15 minutes of collagen/epinephrine injection versus 44% of KO mice. Splenectomy, carried out in KOs, decreased circulating platelet number by approx 40% ($p<0.01$), reverted their hyper-reactive phenotype and protected KO mice against lethal thromboembolism *in vivo*.

In conclusion, COX-2 deletion appears to affect BM megakaryopoiesis, consistently with previous in vitro reports and this appears compensated by enhanced extra-medullary (spleen) megakaryopoiesis. Young and hyper-reactive platelets in KO mice, generated by compensatory spleen megakaryopoiesis, might contribute to increased thrombogenicity *in vivo*.