Platelet release of PDGF causes cyclooxygenase-2 up-regulation in HT29 colon cancer cells: inhibition by imatinib mesylate

M. Dovizio*, S. Alberti*, T.J. Maier,[§] B. Suess,[§] D. Steinhilber[§] and P. Patrignani*

*Department of Neuroscience and Imaging and CeSI, 'G. d'Annunzio' University, Chieti, Italy; [§]Institutes of Pharmaceutical Chemistry and Molecular Bioscience, Frankfurt University, Frankfurt, Germany

Platelet-derived growth factor (PDGF) signaling is considered an important target for cancer treatment (1, 2). In co-cultures of human platelets and HT29 colon cancer cells, we studied the role of PDGF released by platelets and downstream effectors of PDGF receptor (PDGFR) signaling on cyclooxygenase(COX)-2 expression in HT29 cells. Finally, we investigated the effect of imatinib, a PDGFR antagonist on COX-2 overexpression in this setting.

Human HT29 cells ($1x10^6$) were cultured alone or with isolated human platelets ($1x10^8$) up to 20h. PDGF-BB, VEGF, EGF and active TGF-ß1 levels released in the medium were measured by immunoassays. The levels of COX-2 mRNA and protein were assessed in HT29 cultured alone or with platelets, by qPCR and Western blot, respectively. In HT29 cells co-cultured with platelets, we assessed the effects of imatinib (10μM), a PDGF-neutralizing antibody (10μg/ml), wortmannin (PI3K inhibitor, 0.1μM), dm-amiloride [Na^+/H^+ exchanger(NHE) inhibitor, 10μM] and rottlerin (PKCd inhibitor, 10μM) on COX-2 protein induction. Nucleo-cytoplasmic translocation of the mRNA-stabilizing protein HuR was determined by confocal microscopy.

In co-culture of HT29 cells and platelets, we assessed the time-course of the release of different proteins from platelet α-granules. A substantial release of PDGF-BB and TGF-ß1 from platelets began after a lag-time of 4h. However, PDGF-BB levels were higher than those of TGF-ß1 and they continuously increased in a time-dependent fashion up to 20h. In contrast, TGF-ß1, after being released, decreased in a time-dependent manner suggesting its possible cellular re-uptake. At 2-4 h, platelets adhered to HT29 cells and the levels of COX-2 mRNA rapidly increased, and remained stable up to 20h. In contrast, COX-2 protein synthesis began to increase after a lag-time of 8h and, then, it continued to raise, in a time-dependent manner due to platelet-dependent induction of COX-2 mRNA stabilization associated with enhanced cytoplasmic accumulation of HuR. We provide several lines of evidence that platelet PDGF may be involved in posttranscriptional regulation of COX-2: i) the onset of PDGF-BB secretion occurred earlier than that of COX-2 protein synthesis; ii) the time-dependent increase of PDGF-BB levels was accompanied by a parallel upregulation of COX-2 protein; iii) imatinib and a specific anti-PDGF antibody prevented the induction of COX-2 protein; iv) pharmacological inhibition of downstream effectors of PDGFR, i.e. PI3K, NHE, and PKCd, by using wortmannin, dm-amiloride and rottlerin, respectively, reduced COX-2 protein induction.

In conclusion, these results showed that the interaction of platelets with cancer cells led to platelet release of PDGF which contributed to aberrant expression of COX-2. Imatinib affected this program of malignancy triggered by platelet-cancer cell cross-talk. Inhibition of PDGF-dependent induction of COX-2 by the drug may play a role in its anti-cancer efficacy.

References

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