PLATELET-DEPENDENT ACTIVATION OF MYOFIBROBLASTS AND ITS PREVENTION BY SELECTIVE INHIBITION OF PLATELET CYCLOOXYGENASE-1 BY ASPIRIN

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Platelet activation is the first response to tissue damage (Gawaz et al., 2005). However, unrestrained platelet responses in the context of wound healing promote chronic inflammation which may participate in the development of tissue fibrosis and cancer (Gawaz et al., 2005; Dovizio et al., 2017). Activated fibroblasts, which express □-smooth muscle actin (□-SMA), called myofibroblasts, are considered an important cell-type contributing to repair the function of chronically failing organs (Wynn et al., 2012). Growth inhibition and enhanced apoptosis of tissue myofibroblasts have been suggested to be a mechanism for the resolution of the initial injury. The aim of the present study was to verify the influence of platelet-derived products on the phenotype and function of intestinal myofibroblasts in vitro. Thus, co-culture experiments were carried out with human intestinal myofibroblasts (9x104cells) cultured for 24h alone or with isolated human platelets (0.5x108). In the conditioned medium, the concentrations of prostanoids [thromboxane(TX)B2, prostaglandin(PG)E2, PGF2□, PGD2 and 6-keto-PGF1□ (the nonenzymatic metabolite of prostacyclin)] and platelet-derived growth factor(PDGF)-BB and transforming growth factor(TGF)-□1, were assessed by specific immunoassays (Dovizio et al., 2013). In fibroblast lysates, we evaluated the levels of cyclooxygenase(COX)-2 and □-SMA (proteins by Western blot and mRNAs by q-PCR). Moreover, the gene expression profile of mesenchymal markers, including vimentin and fibronectin, and RhoA (RhoA, Ras Homolog Family Member A, involved in cell movement) were assessed by q-PCR. Finally, fibroblast migration was evaluated by scrape wounding assay and cellular proliferation by DNA synthesis-based cell proliferation kit. In some experiments, platelets were pretreated with aspirin (300 □M to completely suppress COX-1 activity) and then the drug was washed away before their incubation with myofibroblasts. In the medium of myofibroblast-platelet co-cultures, a significant ($P<0.01$) increase in prostanoid biosynthesis was detected versus myofibroblasts (<1 ng) and platelets (<1 ng) cultured alone. TXA2 and PGE2 were the most abundant products (250±10 and 66±10 ng, respectively). A significant increase in the levels of PDGF-BB and TGF-□1 (4581±199 and 440±110pg, respectively) versus platelets and myofibroblasts cultured alone ($P<0.01$) was also detected. In co-cultures of aspirin-treated platelets with myofibroblasts, enhanced levels of prostanoids and PDGF-BB and TGF-□1 were reduced by 99%, 40%, and 35%, respectively. However, the incubation of myofibroblasts with platelets was associated with the induction of COX-2, RhoA and mesenchymal markers and the reduction of □-SMA levels. Interestingly, cells changed their shape and appeared elongated by confocal microscopy. They were characterized by enhanced migratory and proliferative capacity. All these changes induced by platelets were significantly abrogated by the incubation of myofibroblasts with aspirinated platelets. Altogether these results suggest that platelets induce signaling pathways which translate into a proinflammatory and mesenchymal/migratory phenotype of myofibroblasts, thus contributing to the development of a chronic wound. Interestingly, selective inhibition of platelet function by aspirin prevented the phenotypic changes

induced by platelet/myofibroblast interaction, thus suggesting an anti-inflammatory and anti-fibrotic action of low-dose aspirin. These effects of aspirin should be verified in vivo in animal models of fibrosis.

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