LIPIDOMICS OF EICOSANOIDS GENERATED BY THE CROSS-TALK BETWEEN PLATELETS AND CANCER CELLS: MODULATION BY CYCLOOXYGENASE INHIBITORS

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CeSI-MET

Eicosanoids are biologically active lipids that have been implicated in tumour development, progression and metastasis (Wang and Dubois, 2010). Among them, cyclooxygenase (COX) and lipoxygenase (LOX) pathways generate prostanoids (PG), leukotrienes (LT) and hydroxyeicosatetraenoic acids (HETEs) (Funk, 2001). The aim of the present study was to characterize the major lipids generated by COX and LOX pathways during the interaction of human platelets with colon cancer cells. Thus, in cocultures of human platelets and human adenocarcinoma cell lines (HT29 and HCA7 cells) we studied: i) the expression of COX-isozymes in platelets and cancer cells, (ii) the lipidomics profile of COX and LOX-derived products by LC/MS/MS. Moreover, we assessed the effect of selective inhibition of platelet COX-1 (by aspirin) and selective inhibition of cancer cell COX-2 (by rofecoxib) on eicosanoid biosynthesis. HT29 or HCA7 cells (2x106) and platelets (2x108) were cultured alone or cocultured for 20 hours. In some experiments platelets were pre-exposed to aspirin (100μM) to completely suppress platelet COX-1 activity, then cells were extensively washed (to eliminate the drug) and added to cancer cells (Dovizio et al. 2013). In addition, rofecoxib (0.3 μM) was used to suppress COX-2 activity. In all experiments, the conditioned media and cell pellets were collected. In the cell pellets, the expression level of COX-1 and COX-2 was assessed by Western blot, while the conditioned medium was assessed for thromboxane(TX)B2 (the hydrolysis product of TXA2), PGE2, PGD2, PGF2α, LTB4, 5-HETE, 8-HETE, 11-HETE, 12-HETE and 15-HETE levels by LC/MS/MS (Aldrovandi et al. 2013). Under baseline condition, HT29 cells expressed both COX-1 and COX-2, while HCA7 cells expressed only COX-2. Platelets expressed COX-1, but not COX-2. The incubation of cancer cells with platelets induced COX-2 expression both in HCA7 cells and HT29 cells. During the coculture of HCA7 and platelets, enhanced generation of TXB2 (74.62±15.27ng/ml), PGE2 (25.14±3.97 ng/ml), PGD2 (18.57±3.13 ng/ml), PGF2α (20.24±2.72 ng/ml) and 11-HETE (0.34±0.07 ng/ml) was observed versus HCA7 cells and platelets cultured alone ($P<0.01$, n=5); 12-HETE levels were significantly higher (96.14±47.40 ng/ml, $P<0.05$, n=5) versus HCA7 cells cultured alone. In the coculture between platelets and HT29 cells, the enhanced generation of TXB2 (88.97±9.00ng/ml), PGE2 (0.45±0.05 ng/ml) and PGF2α (1.13±0.16 ng/ml) ($P<0.01$, n=7) was detected versus HT29 cells and platelets cultured alone; the 12-HETE levels were significantly higher (3.36±0.51 ng/ml, $P<0.01$, n=7) versus HT29 cells cultured alone. In the coculture with HCA7 cells, the pretreatment of platelets with aspirin significantly reduced only the generation of TXB2 (0.99±0.34 ng/ml, $P<0.01$, n=4), while the use of rofecoxib significantly inhibited the levels of PGE2 (3.45±2.51, $P<0.05$, n=4) and PGF2α (1.25±1.13, $P<0.01$, n=3). In the coculture of HT29 cells and aspirin-pretreated platelets the biosynthesis of TXB2 (0.09±0.05 ng/ml, $P<0.01$, n=4), PGE2 (0.14±0.02ng/ml, $P<0.01$, n=4), PGD2 (0.04±0.02ng/ml, $P<0.05$, n=5), and PGF2α (0.09±0.07ng/ml, $P<0.01$, n=5) was reduced. Rofecoxib did not significantly modify the generation of eicosanoids in the HT29 cells-platelet cocultures.

In conclusion, different biologically active eicosanoids are released during the cross-talk between platelets and cancer cells mainly via the activity of COX-isozymes and 12-LOX. Selective inhibition of platelet COX-1 by aspirin or cancer cell COX-2 by coxibs leaves unconstrained the biosynthesis of many biologically active compounds. The coadministration of the antiplatelet agent low-dose aspirin with inhibitors of COX-2 and 12-LOX can translate into improved antitumoral/antimetastatic effects.

References

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