INVOLVEMENT OF RAS IN CALCIUM SIGNALING INDUCED BY LTD_4 IN U937 LEUKEMIA CELLS

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It has been shown that DMSO-differentiated promonocytic cell line U937 (dU937) express high affinity binding sites for LTD_4 and t hat LTD_4 is able to induce $[Ca^{2+}]_i$ transient. We confirmed the presence of $CysLT_1$ receptor by RT-PCR and Western-Blot analysis and we also demostrated that $[Ca^{2+}]_i$ increase induced by 10 nM LTD_4 (fold increase over basal = 3.61 ± 0.19 S.E.M.) was completely inhibited by the Cys-LT_1 receptor antagonists pranlukast (1 μM) and zafirlukast (1 μM). In addition, LTD_4 signal was only partially sensitive to pertussis toxin (-51% at 100 ng/ml, $p<0.01$), but completely inhibited by U-73122 (500 nM) suggesting the involvement of Gi/o proteins and of phospholipase C respectively. ochFuthermore, *Cl. sordelli* lethal toxin (100 ng/ml), a specific inhibitor of Ras family GTPases, was able to inhibit LTD_4-induced $[Ca^{2+}]_i$ increase (-65%, $p<0.01$), while *Cl. difficile* toxin B (30 ng/ml), a specific inhibitor of Rho family GTPases, had no effect.
In line with a supposed role of Ras in LTD_4-induced $[Ca^{2+}]_i$ elevation, treatment of the cells with FTS (dbch100 μM), a potent prenylated Ras methyltransferase inhibitor, also decreased LTD_4 signal (-32%, $p< 0.05$). Finally, using a Ras pull-down assay, we were able to demostrate that LTD_4 induces Ras activation (+122%, $p<0,05$) an effect that is blocked by the two $CysLT_1$ receptor antagonists pranlukast (1 μM) and zafirlukast (1 μM).