## AOX-3 effects, a novel 5-lipoxygenase inhibitor, in breast cancer cells

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**Objective:** It is well documented that leukotrienes (LTs) are involved in the carcinogenesis. Since 5-lipoxygenase (5-LO) is a key enzyme in the synthesis of LTs, we investigated 5-LO expression and examined whether the 5-LO pathway is associated with the proliferation of human breast tumors.
**Methods:** We investigated the potential effects of AOX-3, a selective 5-LOX inhibitor, on human breast cancer cell proliferation and apoptosis, as well as the possible mechanisms involved in the AOX3 mediated effects. In a human breast cancer cell line (MCF-7), cell proliferation and viability were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and the 5-bromo-2'-deoxyuridine (BrdU) assays, respectively. Protein expression was determined by western blot to highlight the key molecular pathways responsible for proliferation, apoptosis, survival and cell cycle arrest. Further, we performed pulse and chase experiments to assess the AOX-3-mediated long term effects on breast cancer cells.
**Results:** Our results showed that MCF-7 cells were highly responsive to AOX-3 administration (IC50 = 0.3875 uM at 48 h). AOX-3 induced cell cycle arrest, suppressed proliferation and induced apoptosis in a dose-dependent manner. In fact we observed a decrease in the Bcl2 and an increase in Bax protein expression levels after treatment, suggesting the possible involvement of AOX-3 in apoptosis. Moreover, MCF7 treated cells showed an increase in the protein expression level of caspase-9, that is the main executor of apoptosis. Moreover, cell proliferation capacity appeared strongly reduced after treatment with 5-LO inhibitor in a dose dependent manner (24h-48h), as revealed by the BrdU incorporation assay. Expression levels of proteins involved in cancer cell proliferation, such as AKT and GSK3β, were reduced, as well. Finally, the pulse-chase assay showed a higher inhibition of cell growth when treatment was repeated every 48 hours for one week. This increase was even more relevant one week after the last treatment.
**Conclusions:** We confirmed the expression of 5-LO in human breast tumors and we demonstrated that AOX-3 was able to suppress tumor growth by inhibiting several pathways involved in proliferation, apoptosis, survival, and cell-cycle arrest. Our results suggest that AOX-3 may be a potential therapeutic target for the control of both the proliferation and the metastatic potential of human breast cancer cells.

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