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Possible Involvement of 15-Deoxy-$\Delta^{12,14}$prostaglandin J_2 in ET-18-O-CH_3-Induced Apoptosis in H-*Ras* Transformed Human Breast Epithelial (MCF10A-*ras*) Cells

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Introduction: It has been known that elevated levels of COX-2 is associated with resistance to apoptosis in cancerous or transformed cells. However, recent studies have shown that up-regulation of COX-2 may be implicated in induction of apoptosis. Previous studies from this laboratory have shown that a novel alkylphospholipid type antitumor agent ET-18-O-CH_3 (1-*O*-octadecyl-2-*O*-methyl-glycero-3-phosphocholine) induces COX-2 expression in H-*ras* transformed human breast epithelial cells (MCF10A-*ras*) while it causes apoptosis in the same concentration range. 15-Deoxy-prostaglandin J_2 (15d-PGJ_2), an endogenous ligand for peroxisome proliferator-activated receptor γ (PPARγ), has been known to possess proapoptotic potential in diverse cell types. In the present work, we have conducted a series of experiments to test the possibility that 15d-PGJ_2 acts as a mediator of ET-18-O-CH_3-induced apoptosis.

Methods: The cell viability was determined by the trypan blue exclusion method. Western blot analysis and RT-PCR were carried out using specific antibodies and primers for COX-2, respectively. The amounts of PGE_2 and 15d-PGJ_2 released into media were measured using the enzyme-immunoassay kits. Apoptosis was assessed by determining proteolytic cleavage of poly(ADP-ribose)polymerase(PARP), caspase-3 activation, and positive TUNEL staining.

Results and Discussion: The addition of a selective COX-2 inhibitor SC-58635 blocked ET-18-O-CH_3-induced apoptosis as well as COX-2 expression and prostaglandin E_2 (PGE_2) production, suggesting that COX-2 induction by this drug is

causally linked to its apoptosis inducing activity. In another experiment, treatment of MCF10A-*ras* cells with 15d-PGJ_2 at 10 μM resulted in apoptotic cell death in 24 h. ET-18-O-CH_3 (2.5 μg/ml) treatment caused release of 15d-PGJ_2 and expression of PPARγ. ET-18-O-CH_3 enhanced the transcriptional activity of PPARγ in MCF10A-*ras* cells transfected with a consensus PPRE construct (pPPRE-luc). Addition of GW9662, a PPARγ antagonist, suppressed the ET-18-O-CH_3-induced COX-2 expression without affecting the cell viability. A kinetic study revealed that ET-18-O-CH_3-induced PPARγ expression preceded the COX-2 induction which was followed by caspase-3 activation through proteolytic cleavage.

Conclusion: We propose that ET-18-O-CH_3 induces COX-2 expression through interaction with PPARγ, stimulating the production of the natural ligand 15d-PGJ_2 which in turn activates PPARγ while inducing apoptosis.