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Red blood cells (RBCs) are known to modify platelet pathophysiology through the release of arachidonic acid (AA) and eicosanoid formation including thromboxane A_2 and thus influence thrombosis and hematosis. Treatment of RBC with a calcium ionophore A23187 could cause a marked enhancement in the release of arachidonic acid in a Ca^{2+}-dependent manner, suggesting that the agent may activate a Ca^{2+}-dependent phospholipase A_2 (PLA_2). A Ca^{2+}-dependent PLA_2 activity was detected in the cytosol of bovine RBC and purified to near homogeneity by sequential uses of chromatographies with ~7,000-fold increase in the specific activity. The purified enzyme migrated as a single band of a molecular weight of 40 kDa on a SDS-PAGE gel. Anti-40 kDa protein polyclonal antibody not only immunoprecipitated the enzymatic activity, but also reacted with the 40 kDa protein in a Western blot analysis, indicating that the 40 kDa protein is the RBC PLA_2. The 40 kDa RBC PLA_2 was characterized as a similar enzyme to Group IV $cPLA_2$, but different in the cross-reactivity with anti-porcine spleen Group IV $cPLA_2$ antibody and the sensitivity to methyl mercury and a newly synthesized quinolone derivative EA4, which has been developed as a selective inhibitor for the 40 kDa RBC PLA_2. Interestingly, pre-treatments of EA4 with human and bovine RBCs markedly attenuated A23187-induced release of AA. Together, our data strongly suggest that the 40 kDa cytosolic form of PLA_2 could be implicated in a Ca^{2+}-dependent physiological release of AA in mammalian RBCs and possibly in thrombotic process in concert with platelets.

[PC1-18] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Effects of endocrine disruptors using mouse mammary gland organ culture system on the formation of preneoplastic lesion

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Endocrine disruptors (EDs) are chemicals which interfere with endocrine system function. These EDs disturb normal endocrine mechanisms and have been observed in nearly all classes of vertebrates. The effects of these EDs are emerged as serious problems on human beings. Especially, as the effects of EDs are reported to be the main causes of hormone-related cancers such as breast cancer among women, we evaluated effects of EDs using mouse mammary gland organ culture (MMOC) model. Originally, the mouse mammary gland in whole-organ culture, an *in vitro* system that is capable of alveolar development differentiation, involution, and oncogenic transformation, has been used to examine the effects of the chemopreventive agents against breast carcinoma. Therefore, we examined that effects of EDs on the formation of preneoplastic lesion in MMOC. The MMOC provides a promising model system to study the mechanisms by which EDs initiate and promote the transformation *in vitro*. Furthermore, inhibition of EDs-induced precancerous lesions in MMOC will be used for evaluating the potential efficacy of chemopreventive agents against breast cancer.

[PC2-1] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

In vitro Inhibitory Effect of the fruits of Citrus aurantium on Rotavirus Infectivity

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