

Red blood cells (RBCs) are known to modify platelet pathophysiology through the release of arachidonic acid (AA) and eicosanoid formation including thromboxane A₂ and thus influence thrombosis and hemostasis. Treatment of RBC with a calcium ionophore A23187 could cause a marked enhancement in the release of arachidonic acid in a Ca²⁺-dependent manner, suggesting that the agent may activate a Ca²⁺-dependent phospholipase A₂ (PLA₂). A Ca²⁺-dependent PLA₂ 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 PLA₂. The 40 kDa RBC PLA₂ was characterized as a similar enzyme to Group IV cPLA₂, but different in the cross-reactivity with anti-porcine spleen Group IV cPLA₂ 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 PLA₂. 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 PLA₂ could be implicated in a Ca²⁺-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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Rotavirus predominantly occurs the sporadic diarrhea in infants and young children. To prevent rotaviral diarrhea, many kinds of orally administered vaccines against each of the epidemiologically important serotypes have been developed. However, the developed vaccines were not complete for preventing the rotaviral diarrhea until now. Therefore, we screened the inhibitory substances from some traditional herbal medicines on the infectivity of rotavirus. Among tested 60 kinds of herbal medicines, the fruit of *Citrus aurantium* had the most potent inhibitory activity on rotavirus infection. The active components of the fruit of *Citrus aurantium* were neohesperidin and hesperidin. Their 50% inhibitory concentrations were 25 and 10 μM , respectively. These active herbal extracts and the isolated active compounds are believed to contribute to the prevention of the rotaviral illness in some degree.

[PC2-2] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Biotransformation of Rhaponticin from the Rhizome of *Rheum undulatum* by Human Intestinal Bacteria and Their Anti-allergic Activity

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During the screening program from discovering hyaluronidase-inhibitory substances from natural products, *Rheum undulatum* (Family Polygonaceae) was found to show inhibitory activity for the activation of hyaluronidase. Rhaponticin isolated from the rhizomes of *Rheum undulatum* (Family Polygonaceae) is metabolized to rhapontigenin and chrysophanol by human intestinal microflora, respectively. Most intestinal bacteria isolated from human feces catalyzed these metabolic pathways. Among rhaponticin and its metabolite, rhapontigenin had the most potent inhibitory activity on a hyaluronidase, a histamine release from mast cell and PCA reaction. The inhibitory activity of rhapontigenin was more potent than that of disodium cromoglycate, one of commercial anti-allergic drugs. These results suggest that rhaponticin in the rhizomes of *Rheum undulatum* should be a prodrug that has an extensive anti-allergic property

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

Two Novel α -L-Rhamnosidase from Quercitrin-hydrolyzing *Fusobacterium* K-60

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Recently we isolated *Fusobacterium* K-60, a bacterium transforming quercitrin to quercetin, from human intestinal microflora. We tried to purify α -L-rhamnosidase from *Fusobacterium* K-60, comparing its properties to those of the previously purified enzymes. *Fusobacterium* K-60 produced two kinds of α -L-rhamnosidases, cytosolic and membrane enzymes. The cytosolic enzyme hydrolyzed naringin and poncirin but not quercitrin. Whereas, the membrane enzyme did vice versa. The cytosolic enzyme was purified to homogeneity by 70% ammonium sulfate fractionation, butyl toyopearl, hydroxyapatite, Sephacryl S-300, Q-sepharose column chromatography. The specific activity of purified α -L-rhamnosidase was 2.89 $\mu\text{mole}/\text{min}/\text{mg}$ protein and its molecular weight was calculated to be 150 kDa by gel filtration. From gel filtration data, it seems to be composed of four identical subunits of 40 kDa with pI and optima pH values of 5.2 and 5.5-7.0, respectively.

[PC2-4] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]