

determined if the two compounds, Zaluzanin-C and Estafiatone isolated from *Anisliaea acerifolia*, modulate iNOS gene expression and PGE₂ synthesis in LPS/IFN- γ -stimulated RAW 264.7 cell. Treatment with two compounds inhibited NO production, PGE₂ synthesis a concentration-dependent manner. Furthermore, two compounds inhibit iNOS protein and mRNA expression. These results of two compounds may provide the possibility for developing anti-inflammatory agents.

[PC1-13] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Development of the On-site Assay for Methamphetamine

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Methamphetamine (MA) abuse has become a serious social concern, particularly in Asia, since it is a potent central nervous stimulant. Confirmation of MA abuse in biological samples has usually been performed using instruments such as GC/MS. It, however, requires great expertise and a considerable amount of time to obtain the result.

For the purpose of fast screening of a large number of samples on the field, we have developed an on-site detection kit based on the membrane immunoassay. Colloidal gold was used as a tracer, and conjugated with the anti-MA antibody (Ab). It was designed so that the Ab-gold conjugate could bind either MA in sample or the MA-BSA conjugate attached to the membrane while it migrate along the strip together with the sample.

The positive/negative result could be read by the naked eye within three minutes without any expertise. The kit developed was allowed to detect MA lower than 1 $\mu\text{g/ml}$ with 150 μl of sample. Evaluation study showed that the strip was stable more than eight months at RT under the desiccated condition. The result of the strip correlated with that of the fluorescence polarization immunoassay by over than 90 %.

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

Regulation of cell growth by transmethylator inhibitor

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Oligosaccharide-linked acyl carrier protein (ACP) purified from porcine liver was identified as a novel transmethylator inhibitor. In cell-free systems, it might act as a noncompetitive inhibitor of the protein carboxyl-O-methyltransferase which methylates the Asp or Glu residue in a large number of proteins. Oligosaccharide-linked ACP is a weak inhibitor of methylation *in vitro*, however, can significantly inhibit the growth of various cancer cell lines including NIH3T3, H-ras-transformed NIH3T3, MDA-MB-231, HT-1376, and AGS. In addition, exposure of H-ras-transformed NIH3T3 with oligosaccharide-linked ACP caused cell cycle arrest at S phase and subsequently cumulative increase of cells at G₀/G₁ phase determined by flow cytometry. Study of this transmethylator inhibitor could be a useful tool for elucidating regulation mechanism of methylation on cell growth.

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

Nitric Oxide inhibits the release of GPI-anchored renal dipeptidase via

extracellular glycosylphosphatidylinositol-specific phospholipase C in porcine renal proximal tubules

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It was previously reported that human urinary dipeptidase was the released form of renal dipeptidase (RDPase) (EC 3.4.13.19), a glycosylphosphatidylinositol (GPI)-anchored glycoprotein to apical membrane of proximal tubules. This *in vivo* release was accomplished by GPI-PLC. In *in vitro* model system of porcine proximal tubules, RDPase was also released by not only bacterial PI-PLC but also endogenous GPI-PLC, which did not follow the intracellular PLC signaling. In this study we assayed the activity of released RDPase using a direct NO donor, sodium nitroprusside (SNP), a nitric oxide synthase (NOS) substrate, L-arginine (Arg), an NOS inhibitor, N ω -Nitro-L-arginine-methyl ester (NAME) in order to investigate the relationship between NO and GPI-PLC activity. Addition of 5 mM Arg to porcine proximal tubules showed the increase of NO production and the decrease of RDPase release simultaneously in the time- and concentration-dependent manner. Treatment with 10 mM NAME inhibited the Arg effect on NO production and RDPase release. A concentration-dependent treatment with SNP also showed the inhibition of RDPase release and 0.5 mM SNP induced an immediate and continuous decrease in GPI-PLC activity. This effect was confirmed quantitatively by western blot using polyclonal antibody raised against porcine RDPase. These results suggest that NO down-regulates the RDPase release by inhibition of GPI-PLC, but not by direct inhibition.

[PC1-16] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Age-related up-regulation of NF-kappaB and modulation by calorie restriction

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Recent data strongly suggested that the calorie restriction (CR) may retard aging by its anti-oxidative action on the regulation of the intracellular redox status. Regulation of the redox-sensitive NF-kB during aging is important because oxidative stress increases during aging, and CR is an effective modulator against oxidative stress. In this present study, we investigated whether age affects the regulation of NF-kB, and how the age effect is modulated by CR. The kidney isolated from Fischer 344 rats at 6, 12, 18, and 24 months of age fed ad *libitum* (AL) and CR rats were used. Results show that the aging process strongly enhanced NF-kB DNA-binding activity that was in parallel with an increased generation of reactive oxygen species (ROS). Accompanied with changes in NF-kB was the decreased inhibitory Ikb α protein in cytosol. At the same time, it was found that the nuclear p65 protein increased with age, affirming the increased translocation of NF-kB into nucleus. However, CR reversed the age-related activation and translocation of NF-kB. Our results further revealed that CR effectively blocked increased activation of NF-kB by suppressing the Ikb α degradation. Based on these data, we concluded that the age-related increase in redox-sensitive NF-kB binding activity is associated with increased ROS, and CR modulates the NF-kB activation by suppressing oxidative stress.

[PC1-17] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

A 40 kDa Ca²⁺-dependent Cytosolic Phospholipase A2 Is Implicated in A23187-induced Release of Arachidonic Acid from Mammalian Red Blood Cells

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