parallelism of the log dose-response lines. It was found that there was no significant difference in the potency between the two analytical methods. The weighted geometric mean of each of the laboratory geometric mean (95% confidence interval) was 3,370,219 IU (3,265,821~3,477,954) by the participants' own methods and 3,289,187 IU (3,202,000~3,330,752) by parallel line analysis.

[PC1-45] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Effect of lawsone methyl ether on the release of glycosylphospatidylinositol-anchored renal dipeptidase and alkaline phosphatase

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Detergent-insoluble microdomains (rafts) may play crucial roles in many cellular functions such as membrane trafficking, cell signaling and human diseases. The variant surface glycoproteins are localized in microdomains and anchored to the cell surface via a glycosylphosphatidylinositol (GPI) anchor. Renal proximal tubules (PTs) were used to compare the release between GPI-anchored renal dipeptidase (RDPase, EC 3.4.13.19) related with cellular signaling pathway and GPI-anchored alkaline phosphatase (APase EC 3.1.3.1) of ubiquitous location. Cell viability was evaluated using a MTT assay and was shown to be intact. The RDPase was released by endogenous GPI-specific phospholipase C (GPI-PLC). Although APase is also a target of GPI-PLC, it showed different activity as compared with RDPase when released from renal PTs in the presence of lawsone methyl ether (LME), a naphtoquinone compound isolated from a Tai medicinal plant. Incubating PTs with various concentration of LME, RDPase activity was increased to 300% (500μM LME) of the control whereas APase activity was not affected significantly. Such increase was also confirmed as a function of time. The results suggest that the release mechanism of RDPase was different from that of APase.

[PC1-46] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Retrovirus-mediated Gene Delivery of TIMP-2 Inhibits Invasion, Migration and Angiogenesis

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The matrix metalloproteases (MMPs) play important roles in invasion, metastasis and angiogenesis in various cell types. An endogenous inhibitor of MMP, tissue inhibitor of metalloprotease-2 (TIMP-2), has high specificity for MMP-2. An imbalance between MMP-2 and TIMP-2 causes the degradation of the extracellular matrix associated with pathological events including invasion, metastasis and angiogenesis. Since TIMPs are secreted molecules, they have the potential to be used for gene therapy of certain tumors. In the present study, we have studied the retrovirus-mediated delivery of TIMP-2 in H-ras MCF10A cells in which MMP-2 was shown to be responsible for the H-ras-induced invasive phenotype. Recombinant retrovirus containing TIMP-2 gene was used to infect PG13 cells (packaging cell line). When the H-ras MCF10A cells were treated with the conditioned media of PG13/TIMP-2, a dose-dependent inhibition of MMP-2 secretion was observed by gelatin zymography. TIMP-2 overexpression mediated by retrovirus significantly reduced the invasiveness and migration of H-ras MCF10A cells in a dose-dependent manner. In addition, retroviral delivery of TIMP-2 efficiently inhibited angiogenesis of HUVEC cells in a dose-dependent manner as evidenced by in vitro tube formation assay. Taken together, we show that the down-regulation of MMP-2 by TIMP-2 overexpression inhibits invasive and migrative properties of H-ras MCF10A cells and angiogenesis of HUVEC cells. Our data showing efficient inhibition of cancer progression by retrovirus-mediated delivery of TIMP-2 suggest a possible application for gene therapy to prevent and treat cancer.

[PC1-47] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Development of Immunocomplex Reagent for One-step Fluorescence Polarization Immunoassay of DDT

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DDT and its related metabolites (DDA, DDE, DDD) were investigated using a homogeneous fluorescence polarization immunoassay (FPIA). Fluorescence polarization immunoassay (FPIA) for DDT was developed using a fluorescence polarization analyzer in photo check mode. FPIA is based on the increase in fluorescence polarization of a small fluorescent-labeled tracer when it was bound by specific antibody. If the sample contains DDT, tracer will compete with DDT for the antibody binding and the polarization signal will decrease.
Nine fluorescence-labeled DDT tracers were synthesized and characterized by the combination of three DDT derivatives, DDA, DDHP and DDT7, and three fluorescence labels, fluoresceinamine isomer I (AF1) and II (AF2), and ethylenediamine fluoresceinthiocarbamyl (EDF). The bindings of tracers with specific DDT antibody produced from DDT7-KLH immunogen were investigated to select optimal pair of tracer and antibody. Significant differences were found in titer level, sensitivity, and assay kinetics with pairs of various combination. Among them, a pair of DDT7 and AF2 tracer (Rf=0.3 in $CHCl_3$:MeOH, 4:1) showed best response.
To simplify the FPIA procedure, the immunocomplex reagent, that is a pre-equilibrated mixture of antibody and tracer, was prepared. This immunocomplex could be used as one direct single reagent for the measurement of displacement of tracer from immunocomplex after sample addition. Thus, we could measure a fluorescence polarization of DDT analyte with only one-step addition of sample without incubation. The detection limits of DDT, DDE and DDD by FPIA in optimal immunoreagents and condition is approximately 10 ng/ml for DDT derivatives using 50 ul samples within 7 minutes. DDA is 100 times less sensitive. The immunocomplex reagent has proven to be significantly stable comparing with respective solution of antibody and tracer.

[PC1-48] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

AgingPath : Database programmed to investigate aging process

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Aging is an inevitable biological process that affects all living organisms. The process is time-dependent and inevitably leads to a functional decline. Underlying pathophysiologic process may best be explained by considering several biologic processes. Programmed genetic processing (e.g. apoptosis), oxidative stress, concomitant disease process, and factors not yet identified may all work to determine the rate and rapidity of aging. We programmed an aging regulatory pathway database (AgingPath) based on known biomolecules that have a role in aging, in order to better understand the process. In addition, beneficial effect of how caloric restriction (CR) may work to slow this process is also investigated. AgingPath is divided into two main sections, A) list of biomolecules that vary with aging, and B) list of various biomolecules which are modulated by CR. Currently, AgingPath is further divided into five different categories, under each category, search function is available. Many diagrams or graphic figures contain hot-links, which when activated, result in more detailed information. Pre-defined users (data entry person) are able to submit a new biomolecule or edit an existing biomolecule to reflect a latest development. AgingPath, with latest updated information, can help find a new biomarker. Similarly, the mechanism of CR on slowing of aging process may better be defined. AgingPath is freely available at http://pro.bio.pusan.ac.kr.

[PC1-49] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]