Lee, S. K., Kim, S.#, Kim, M. Y., and Chun, Y. J.

College of Pharmacy, Chungang University, Seoul 156-756; #Natural Products Research Institute, Seoul National University, Seoul 110-460

Recently we have suggested that various hydroxystilbene compounds from natural sources showed strong inhibition of activities of human P450 1 isozymes such as CYP1A1, 1A2, and 1B1 (Chun, Y. J., Ryu, S. Y., Jeong, T. C., and Kim, M. Y. Drug Metab. Dispos. 29:1-6, 2001). Here we reported that 3,3',4,5,5'-pentamethoxystilbene (PMS), a synthetic stilbene compound, exhibited a potent and selective inhibition of human CYP1A1 with an IC50 value of 0.14μM. PBS showed 6700-fold greater selective inhibition of CYP1A1 over CYP1A2 (IC50=934μM) and 23-fold selectivity for CYP1A1 over CYP1B1 (IC50=3.2μM). PMS did not show any significant inhibition of ethoxyresorufin O-deethylation (EROD) activity in human liver microsomes. To elucidate the mechanism of inhibition by PMS, kinetic studies were performed. Analysis of the mode of inhibition indicated mixed-type inhibition of CYP1A1. The inhibition by PMS was not mechanism-based. The trapping agents glutathione, N-acetylcysteine, or dithiothreitol prevented the inhibition. Taken together, PMS is one of the most selective inhibitor of human CYP1A1 and may be considered as a good candidate for a cancer preventive agent in human.

[PC1-2] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Identification of a nucleolus protein, hNopp140, as a specific binder to doxorubicin by an affinity selection method with a phage display library

Jin YN, Yu YG

Structural Biology Center, Korea Institute of Science and Technology

Doxorubcin is a widely used anti-cancer drug that has cytotoxic activity against various types of cancer cells. DNA intercalation was assumed as one of the mechanism of the drug, however, the precise target and the mechanism of cytotoxicity of doxorubicin were not fully revealed. To examine the potential target protein against doxorubicin, we have used a biopanning method with a T7 phage library expressing human liver cDNA on the surface of phage. The phage library was screened against the immobilized doxorubicin, and a phage clone was isolated. Sequence analysis showed that the cloned phage displayed the C-terminal region of hNopp140 that had an important role in the biogenesis of nucleolus as well as cell division. When the cloned region of hNopp140 was expressed in E. coli and purified, it could be phosphorylated by casein kinase II and oligomerized in the presence of magnesium and fluoride ions as in vivo state. In addition, it interacted specifically to doxorubicin with apparent dissociation constant of 4.5 ´ 10-6M. Interestingly, doxorubincin bound to only the native form of purified protein not to the phosphorylated form. The significance of the interaction between doxorubicin and hNopp140 with relation to the cytotoxic activity of doxorubicin was discussed.

[PC1-3] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Proteomic study of ginsenoside-Rg1 (G-Rg1) in NIH3T3 mouse fibroblast cells.

Choi JK, Yang YI, Lee JK, Yoo GS

College of Pharmacy, Chonnam National University, Kwangju 500-757, South Korea

We have studied an activation mechanism of pp60c-src protein tyrosine kinase (PTK) by ginsenoside-Rg1 (G-Rg1) in NIH3T3 mouse fibroblast cells using proteomic technique. It was previously reported that G-Rg1 stimulated the activation of c-src kinase at 20 M with a 18hr-incubation, increasing the activity by 2-4-fold over that of untreated control with an increased cell proliferation. In the present study, we examined effects of G-Rg1 on pp60c-src protein tyrosine kinase(PTK) activity using a 2D

protein mapping. According to the results, 1) Western blot analysis shows that pp60c-src kinase is activated by new phosporylation of the protein shifting the protein from lower band (inactivated form) to upper band (activated form). 2) 2D protein map analysis reveals that G-Rg1 treated total cell protein has increased protein expression in 12 different proteins including src kinase.
Taken together, these results suggest that the activation of c-src kinase by G-Rg1 is caused by an increase in the specific activity of the kinase. We suggest that ginsenoside Rg1 may lead to cell proliferation via the activation of cellular signal transduction pathway involving pp60c-src kinase.

[PC1-4] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Costunolide Induces Mitochondrial Permeability Transition and Cytochrome C Release Through ROS Generation

Lee HJ, Lee KT1, Park HJ2, Chi SG and Park JH

Department of Pathology, College of Medicine and 1College of Pharmacy, Kyung Hee University and 2 Division of Applied Plant Science, Sangji University

Costunolide is an active compound isolated from the root of *Saussurea lappa* Clarks, a Chinese medicinal herb, and is considered a therapeutic candidate for various types of cancers. Nevertheless, the pharmacological pathways of costunolide are still unknown. In this study, we investigate the effects of costunolide on the induction of apoptosis in HL-60 human leukemia cells and its putative pathways of action. Using apoptosis analysis, measurement of reactive oxygen species (ROS), and assessment of mitochondrial membrane potentials, we show that costunolide is a potent inducer of apoptosis, and facilitates its activity via ROS generation, thereby inducing mitochondrial permeability transition (MPT) and cytochrome c release to the cytosol. ROS production, mitochondrial alteration, and subsequent apoptotic cell death in costunolide-treated cells were blocked by the antioxidant N-acetylcystein (NAC). Cyclosporin A, a permeability transition inhibitor, also inhibited mitochondrial permeability transition and apoptosis. Our data indicate that costunolide induces the ROS-mediated mitochondrial permeability transition and resultant cytochrome c release. This is the first report on the mechanism of the anticancer effect of costunolide.

[PC1-5] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Intermedeol Induces Differentiation and Apoptosis of Human Leukemic cell HL-60

Jeong SH, Koo SJ, Park HJ**, Lee KT*

Department of Food & Nutrition, *College of pharmacy, Kyung-Hee University, Seoul,130-701, ** Department of Botanical Resources, Sang-Ji University, Wonju, 220-702

Intermedeol, a sesqueterpene isolated from Ligularia Fischery var., has a antitumor activity by induction of cell differentiation and apoptosis in HL-60. Intermedeol inhibited cell proliferation in a dose- and time-dependent manner and induced differentiation toward granulocyte and monocyte/macrophage lineages. Markers of differentiation, NBT reduction and expression of CD14 and CD66 surface antigens, were significantly increased in dose-dependent manner. Concentration of Intermedeol >40㎍/㎖ rapidly induced apoptosis. These apoptotic features were identified by increasing of hypodiploid nuclei and early phosphatidylserine externalization. These events were accompanied by a decline in the expression of c-Myc and Bcl2 protein. These results suggest that Intermedeol induce differentiation and apoptosis in HL-60 cells

[PC1-6] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]