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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

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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.

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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