We present an automatic method for docking inhibitors into enzyme binding sites with computer docking program, QXP(Quick eXPlore). Its search algorithms are derived from the method of Monte Carlo perturbation with energy minimization in Cartesian space. This program is reliable, easy to use and sufficiently rapid for full conformational search for flexible cyclic and acyclic molecules. In this study, inhibitors have been docked into dihydrofolate reductase of different species which plays an important role in the process of DNA replication and is therefore a target for anticancer, antibacterial and antifungal drugs. Docking searches of the energy minimized inhibitors have given rms differences between the docked structures and the X-ray crystal structures, of 0.06 to 1.5 Å. It has also demonstrated that a new inhibitor with biological activity proven experimentally docks well into the active site of the enzyme. The results serve to confirm the reproducibility of the program of the X-ray structures and to provide binding modes for new inhibitors to the target enzymes.

[PC1-11] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Development of a dry immunotest strip to detect tetrahydrocannabinoid

Choi JE, Lee JR, Choi MJ

Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, Seoul, Korea

A dry immunochromatography strip was developed to detect tetrahydrocannabinol (THC), a major active ingredient of marijuana. △8-THC-BSA was used as a detection probe on the nitrocellulose membrane where BSA was linked at 8 position of THC. Protein A purified anti-THC monoclonal antibody (isotype: IgG1) was labeled with colloidal gold and the antibody-gold conjugate as a tracer was applied on the glassfiber membrane.
Antibody was titrated to find proper coating concentration of THC-BSA on the microtiter plate and the THC binding reactivity of antibody was tested using a competitive inhibition test with samples containing known amounts of THC. The ELISA result showed the THC standard curve in the range of 2~200 ng/ml with 1 ug/ml of coating concentration.
For the dry immunochromatography test strips, a antibody-gold tracer was tested using △8-THC-BSA on the result line to find the resulting line formation by the antigen-antibody reaction. At the optimized condition, the result indicates that the test strip could detect 1 ug/ml of THC in urine.

[PC1-12] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

thiols / Fe2+ system-mediated Oxidative Inactivation of brain ecto -5'-nucleotidase

Liu XW°, Sok DE

college of pharmacy, chungnam university

Ecto-5'-Nucleotidase, was purified from bovine brain membranes, and
subjected to oxidative inactivation. The 5'-nucleotidase activity decreased slightly after the exposure to either glutathione or Fe^{2+}. The glutathione-mediated inactivation of 5'-nucleotidase was potentiated remarkably by Fe^{2+}, but not Cu^{2+}, in a concentration-dependent manner. Similarly, glutathione exhibited a concentration-dependent enhancement of the Fe^{2+} of an intermediary role of superoxide ions or H_2O_2 in the action of glutathione/Fe^{2+} system, superoxide dismutase and catalase expressed a substantial protection against the inactivation by the glutathione/Fe^{2+} system. Meanwhile, hydroxyl radical scavangers such as mannitol, benzoate or ethanol were incapable of preventing the inactivation, excluding the participation of extraneous

hydroxyl radicals. Whereas adenosine 5'-monophosphate as substrate exhibited a modest protection against the glutathione/Fe^{2+} action, a remarkable protection was expressed by divalent metal ions such as Zn^{2+} or Mn^{2+}. Structure-activity study with a variety of thiols indicates that the inactivating action of thiols in combination with Fe^{2+} resides in the free sulfhydyl group and amino group of thiols. Overall, thiols, expressing more inhibitory effect on the activity of 5'-nucleotidase, were found to be more effective in potentiating the Fe^{2+}-mediated inactivation. These results suggest that ecto-5'-nucleotidase from brain membrane is one of proteins susceptible to thiols/Fe^{2+}-catalyzed oxidation. The work was partly supported by Korea Research Foundation (1998-001-F00772).

[PC1-13] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Chemopreventive effect of capsaicin in SK-Hep-1 hepatocellular carcinoma cell line

Chung MY, Kang HJ, and Moon A

College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea

Hepatocellular carcinoma is one of the most lethal malignancies and there is no effective preventive measure in this highly malignant disease to date. In the present study, we investigated the chemopreventive potential of capsaicin (8-methyl-N- vanillyl-6-nonenamide), the principal pungent ingredient found in hot red pepper, in SK-Hep-1 hepatocellular carcinoma cells. Treatment of capsaicin inhibited growth of SK-Hep-1 cells in a concentration-dependent manner, with an IC_{50} value of 119 uM. Methoxy-capsaicin was less potent (IC_{50} of 264 uM), indicating that the hydroxyl group of capsaicin is important in growth-inhibitory property of capsaicin. This study reveals that the inhibitory effect of capsaicin on SK-Hep-1 cell growth is mainly due to the induction of apoptosis as evidenced by DNA fragmentation and nuclear condensation. In order to investigate the molecular mechanisms of capsaicin-induced apoptosis, we examined the effect of capsaicin on anti-apoptotic Bcl-2 and pro-apoptotic Bax levels. We show that capsaicin prominently reduced the ratio of Bcl-2 to Bax which may trigger apoptosis in SK-Hep-1 cells. We also show that caspase-3 activity may be involved in capsaicin-induced apoptosis. These results demonstrate that capsaicin efficiently induced apoptosis in SK-Hep-1 cells, suggesting an effective strategy for hepatocellular carcinoma chemoprevention.

[PC1-14] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Induction of apoptosis by 3,4'-dimethoxy-5-hydroxystilbene in human myeloid leukemic HL-60 cells

Young Jin Chun°, Sung Hee Lee, Shi Yong Ryu†, Han Bok Kim‡, and Mie Young Kim

College of Pharmacy, Chungang University, Seoul 156-756; †Korea Research Institute of Chemica Technology (KRICT), Taejon 305-600; ‡Department of Life Science, Hoseo University, Asan, Chungnam 336-795.

3, 4···-Dimethoxy-5-hydroxystilbene (DMHS) is a hydroxystilbene compound obtained by methylation and acid hydrolysis of piceid (resveratrol-3-O-glucoside) from *Polygonum cuspidatum*. Herein, we report that DMHS induces programmed cell death or apoptosis in human promyelocytic leukemic HL-60 cells. We found that treatment of HL-60 cells with DMHS suppressed the cell growth in a concentration-dependent manner with IC_{50} value of 25 uM. DMHS increased the apoptosis characterized by internucleosomal DNA fragmentation and nuclear condensation. The cell death by DMHS was partially prevented by the caspase inhibitor, zVAD-fmk. DMHS caused activation of caspases such as caspase-3, -8, and -9. Immunoblot experiments revealed that DMHS-induced apoptosis was associated with the