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The proteolysis of HCV non-structural protein is reported to be the most essential process for HCV virus replication. This proteolytic processing is catalyzed by a chymotrypsin-like serine protease which is located in the N-terminal region of non-structural protein 3(NS3). The cDNA of HCV NS3 (1-180) protease was cloned into expression vector. The fusion protein with the N-terminal six histidine was over-expressed in Escherichia coli. In order to discover NS3 protease inhibitors, we have established a high throughput screening(HTS) system based upon a fluorogenic assay in a 96-well format. Over 4,000 compounds in-house library were evaluated for their inhibitory activities on HCV NS3 protease with newly developed HTS method. Among these compounds, 35 compounds were founded with IC50 values of less than 5 uM and one compound with less than 1 uM. The advantages of this fluorogenic NS3 protease assay system are fast, accurate and reproducible.
[This study was supported by a grant of the National R&D Program, Ministry of Science & Technology, Korea (CH1-3-12)]

[PA1-4] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

INFLUENCE OF DOXORUBICIN ON CATECHOLAMINE SECRETION FROM THE PERFUSED RAT ADRENAL GLAND

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Doxorubicin (DX, adriamycin) is an anthracycline that is a highly effective chemotherapeutic agent used largely in the treatment of solid tumors (Singal and Iliskovic, 1998, Feldman et al., 2000, Slamon et al., 2001). Bounias and his coworkers (1997) have shown that catecholamines (CA) including epinephrine, norepinephrine and dopamine, and DOPA enhance the generation of hydroxyl radicals by chemotherapeutic antibiotics (DX, farnorubicin and mitomycin C). It has been also found that in closed-chest pure-bred beagles infused with DX into coronary artery, the plasma norepinephrine concentration as well as plasma natriuretic peptide levels were greatly increased. Increased circulating and heart CA levels have been reported in experimental animals treated with DX or daunorubicin, a closely related anthracycline (Bristow et al., 1979, Bristow et al., 1981, Soldani et al., 1981). Moreover, at a lower concentration (3 x 10-6 M), DX facilitated CA secretion induced by acetylcholine and 51mM K+ from the bovine adrenal medulla (Pinto et al., 1987). In contrast with these results, Robison and Girl (1987) have reported that plasma CA and myocardial guanylate cyclase activity examined at 14 weeks after treatment with DX in rats were unchanged throughout the course of the study. In acute and chronic studies treated with DX, in rabbits, myocardial CA levels were also unchanged (Jackson et al., 1984). On the other hand, it has been shown that chronic adriamycin treatment rather inhibits the neuronal exocytotic release of CA at the cardiac sympathetic nerve terminals of the rabbits (Kawada et al, 2000). Therefore, the present study was attempted to investigate the effect of doxorubicin on secretion of catecholamines (CA) evoked by ACh, high K+, DMPP and McN-A-343 from the isolated perfused rat adrenal gland and to establish the mechanism of its action. Doxorubicin (10-7 ~ 10-6 M) perfused into an adrenal vein for 60 min produced dose- and time-dependent inhibition in CA secretory responses evoked by ACh (5.32 x 10-3 M), DMPP (10-4 M for 2 min) and McN-A-343 (10-4 M for 2 min). However, doxorubicin did not affect CA secretion by high K+ (5.6 x 10-2 M). Doxorubicin itself did also fail to affect basal catecholamine output. Furthermore, in adrenal glands loaded with doxorubicin (3 x 10-7 M), CA secretory responses evoked by Bay-K-8644, an activator of L-type Ca2+ channels and cyclopiazonic acid, an inhibitor of cytoplasmic Ca2+-ATPase were time-dependently inhibited. However, daunorubicin (3 x 10-7 M), given into the adrenal gland for 60 min, attenuated CA secretory responses evoked by ACh (5.32 x 10-3 M), DMPP (10-4 M for 2 min) and McN-A-343 (10-4 M for 2 min), not that by high K+ (5.6 x 10-2 M). Taken together, these results suggest that doxorubicin inhibits greatly CA secretion evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors, but does not affect that by membrane depolarization. It is thought that this inhibitory effect of doxorubicin may be mediated by blocking the calcium influx into the rat adrenal medullary chromaffin cells as well as by the inhibition of Ca2+ release from the cytoplasmic calcium store. It also seems that there is no difference in the mode of action between doxorubicin and daunorubicin in rat adrenomedullary CA secretion.

[PA1-5] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

In Vitro Anti-tumor Activity of Novel Farnesyltransferase Inhibitor

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Traditionally, cancer chemotherapy have focused on cytotoxic intervention at the level of DNA replication. While cytotoxic agents have shown limited efficacy against rapidly growing tumor cells, they cause serious toxic and side effects due to attack against both normal and neoplastic cells without distinction. Since the role of oncogenic ras protein in human tumors have been dicovered, medicinal chemists have paid their attention to the ras activation catalyzed by farnesyltransferase in the hope of developing cancer cell-specific agent without toxicity on normal cells. YH3939 inhibited farnesylation of H-ras and K-ras4B by purified human farnesyltransferase with IC50 values of less than 1.0 nM. Enzyme kinetic studies of YH3939 have demonstrated that it is competitive with respect to ras protein. YH3939 showed potent inhibition on anchorage dependent and independent soft agar growth of human tumor cells which express mutant K-ras. Furthermore, the processing of oncogenic ras in K-ras4B transformed fibroblast and A549 human lung tumor cell lines was disrupted by YH3939. This accounts for the ability of YH3939 to inhibit tumor cell growth and to abolish the malignancy of cancer cells by blocking oncogenic Ras activity. Therefore, our findings indicate that YH3939 is a potent inhibitor of Ras processing with robust anti-tumor properties.
[This study was supported by grant of the Good Health R & D Project, Ministy of Health welfare, Korea (HMP-98-D-7-0010)]

[PA1-6] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Antioxidative Effect of In Rat Hippocampal Slice, Compound SY-013, A New Stilbene Derivative.

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Resveratol (trans-3,4',5-trihydroxystilbene) is naturally occuring phytoalexin found in grapes. This ingredient was found to act as a antioxidant agent, anticancer agent and cardiovascular disease drug. Recently, It is feasible to study possible neuroprotective effect of resveratrol against neural injury through chronic administration of the compound to experimental animals. But, Virgili et al reported that resveratrol have not significant degree of neuroprotection because of resveratrol structure being high polar. Our study was designed to search for alternative materials like resveratrol derivatives, which having non polar, high bioactivities. SY-013 (Compound I), which is resverarol derivatives, with a lower polarity than resveratrol for Compound I was synthesized by single step process. To evaluated which Compound I could exert the protective effects on ischemic-induced neuronal damage, Compound I were treated to the reaction medium from the hipocampal slice in ischemic condition. Also It was studied for whether it is directly associated radical scavenge activity.
Our results suggest the compound I has exerted to prevent loss of ATP under ischemic condition in the hippocampal slice. And It suppressed the increase on the radical producing in PC12 cell line.

[PA1-7] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

OST-3964, A Human Cathepsin K Inhibitor, Inhibits Bone Resorption In Vitro

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Cathepsin K is a cystein protease that plays an essential role in osteoclast-mediated degradation of organic matrix of bone. This enzyme promises the future therapy for the excessive bone resorption such as