

We investigated the alteration of signal transduction on VIP-induced relaxation in cat esophagitis. Acute esophagitis (AE) was induced by perfusion with 0.1N HCl at a rate of 1 ml/min for 45 min over three consecutive days. We have isolated smooth muscle cells of esophagus by enzymatic digestion with collagenase F.

After pretreatment of ACh, we compared relaxation of normal cells with those of esophagitis. VIP produced dose-dependent relaxation in normal cells, and this relaxation curve was down shifted when compared with those of esophagitis cells. SNP or SIN-1, which is a NO donor, produced the dose-dependent relaxation in normal cells, but there is no difference as compared with esophagitis. Forskolin (cAMP activator) or db-cAMP (cAMP analog) produced dose-dependent relaxation in normal cells, and this relaxation curve was down shifted when compared with those of esophagitis cells. The relaxation of esophagitis cells is reduced by 20% as compare with normal cells. 8-Br-cGMP (cGMP analog) induced dose-dependent relaxation, but there is no difference between normal and esophagitis.

This result suggests that cAMP dependent pathway rather than cGMP dependent pathway plays a role on the regulation of VIP induced relaxation in cat acute esophagitis.

[PA1-49] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Novel ginseng saponine metabolite induces apoptosis through activation of caspase-8, BID cleavage and cytochrome c release in HepG2 cells

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The novel intestinal bacterial metabolites of ginseng protopanaxadiol saponins 20-O-(β -D-glucopyranosyl)-20(S)-protopanaxadiol (IH-901) formed from ginsenosides Rb1, Rb2 and Rc, is reported to be a potential chemopreventive and chemotherapeutic agent. We show here that IH-901 induced apoptosis in human hepatoblastoma HepG2 cells as determined by morphological analysis, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining, DNA fragmentation and flow cytometric analysis. The apoptosis by IH-901 induced through mitochondrial pathway involving caspase-8, Bid cleavage, cytochrome c (cyt c) release and caspase-3 activation. Caspase activation was a necessary requirement for apoptosis by IH-901 because the pretreatment with the broad-spectrum caspase inhibitor (zVAD-fmk, 50 μ M) and specific caspase-8 inhibitor (zIETD-fmk, 10 μ M) for 18h increased cell viability to 55 % and 47 %, 1.7- or 1.5-fold compared with the IH-901 only ($p < 0.01$, Student t-test). The decrease in the cell death by pretreatment with antagonistic anti-Fas antibody (ZB4) and the activation of the initiator caspase-8 indicated that IH-901 induced signaling pathway requires the Fas death receptor. Though IH-901 did not induce Fas or FasL mRNA and protein expression, it appeared that the cleavage of cytosolic BID by caspase-8 to truncated tBID. tBID translocated to the mitochondria to induce the oligomerization results in the cytc release in a time-dependent manner, whereas antiapoptotic mitochondrial Bcl-x decreased in a time-dependent manner. Primary hepatocytes isolated from normal Sprague-Dawley rats are not affected by IH-901 (60 μ M). The very low toxicity in normal hepatocytes and its high activity in hepatoblastoma HepG2 cells suggest that IH-901 is a promising experimental cytotoxic agent.

Our results indicated that IH-901 induces apoptosis through caspase-8, BID cleavage, cyt c release, caspase-3 and PARP activation. These results also suggest that oligomerization of tBID plays a critical regulator the release of cytc.

[PA1-50] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Ginsenoside-Rh1 and Ginsenoside-Rb1 display estrogenic activity in human breast carcinoma MCF-7 cells.

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One of the action mechanisms for ginsenoside activity involves binding to intracellular steroid receptors that act as transcriptional factors in the nucleus. We have examined the possibility that the components of Panax ginseng, ginsenoside (G)-Rb1, G-Rc, G-Re, G-Rf, G-Rh1, and G-Rh2, act by binding to the steroid hormone receptors, estrogen (ER), glucocorticoid receptor (GR), androgen receptor (AR), and retinoid receptor (RAR). Both G-Rb1 and G-Rh1 activated transcription of estrogen-responsive luciferase reporter gene in MCF-7 breast cancer cells and CV-1 kidney fibroblast cells transiently transfected with ER α or ER β at 10 μ M concentration. This activation was inhibited by specific estrogen antagonist, ICI 182, 780. We next examined whether G-Rb1 and G-Rh1 activate an endogenous estrogen-responsive gene. G-Rb1 and G-Rh1 increased the expression of estrogen-responsive gene, C-fos at the mRNA level in MCF-7 cells at 24 h of treatment as measured by quantitative reverse transcriptase-polymerase chain reaction. But screened none of the above ginsenosides including G-Rb1 and G-Rh1 did not activate glucocorticoid receptor, androgen, or retinoid receptor in CV-1 cells transiently transfected with steroid hormone receptors and hormone-responsive reporter plasmids. These data supported the specificity of G-Rb1 and G-Rh1 acting through the ER. Taken together, these results demonstrated that G-Rb1 and G-Rh1 are weak phytoestrogen acting via ER, not glucocorticoid receptor, androgen receptor, and retinoid receptor. This work was supported in part by grants from the Korean Ministry of health and welfare (HMP-00-O-21600-0009, YJL).

[PA1-51] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Effect of DIDS on Lead Transport into astrocytes

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We have previously reported some results which lead uptake into astrocytes increased time- and concentration-dependently, and under different pH conditions, levels of lead uptake were greatly different. Divalent metal transporter 1 (DMT 1) is not major route although it is involved in lead uptake into astrocyte. Levels of lead uptake at pH 7.4 were 10 times more higher than at pH 5.5. In this study we investigated what kind of transport system mediate transport of lead into astrocytes. We did effects of inhibitors of anion exchange or H⁺ co-transport on lead uptake into astrocyte in different pH condition. We used 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid, disodium salt (DIDS), Furosemide, Probenecid, Cyano-hydroxycinnamic acid (CHCA) and Niflumate as a inhibitor. Immortalized human fetal astrocyte (SV-FHA) cells were cultured in medium containing Dulbecco's modified Eagle's medium and added with inhibitors 15 minutes before lead treatment. Lead uptake assay was done in incubation condition of pH 5.5 and 7.4. Lead uptake into astrocytes increased time-, pH-, and concentration-dependently, and was saturable. At pH 7.4 lead uptake was the highest level, and only DIDS inhibit lead uptake but others did not. At pH 5.5 DIDS increased lead uptake. Lead uptake was inhibited by DIDS in dose-dependent manner and done 75% in 100 μ M of DIDS. We are investigating about the mechanism of inhibition of lead uptake induced by DIDS.

[PA1-52] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Effects of extremely low frequency magnetic field on generation of hydroxyl radical

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It has been reported that extremely low frequency magnetic field (ELF-MF) produced increase of lipid peroxidation in vivo. In this study, we examined the effect of ELF-MF on generation of hydroxyl free radical in vitro. Hydroxyl radical is produced by incubation of FeCl₃ and H₂O₂ in Tris-buffer solution. The generation of hydroxyl radical during sham or exposure to ELF-MF was examined by measuring salicylic acid hydroxylation adducts, 2,3-dihydroxylbenzoic acid (DHBA) and 2,5-DHBA using HPLC-electrochemical detector system. Exposure conditions were changed in time (15 min, 30 min) and intensity of ELF-MF exposure (50 V, 100 V, 150V) to find the dependence on them of hydroxyl radical generation. The production of hydroxyl radical was elevated in 15 min exposure to 50 V, 100 V but not 150 V ELF-MF. The increased hydroxyl radical concentration, however, was also found in 30 min exposure to 150 V ELF-MF. Comparing the generation of hydroxyl radical relevant to ELF-MF exposure time, the significant increase of