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-(ß-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 uM) and specific caspase-8 inhibitor (zIETD-fmk, 10um) 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 (60uM). 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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