The mechanism of ET-1 or C2-ceramide-induced the contraction of circular smooth muscle cells in cat esophagus

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We examined the mechanism of C_2 or ET-1-induced contraction of circular smooth muscle cells in cat esophagus. C_2 or ET-1 produced the contraction of smooth muscle cells isolated by enzymatic digestion with collagenase in a concentration-dependent manner. The pertussis toxin (PTX) inhibited the contraction induced by ET-1. The Gi3 or Gβ antibody inhibited the contraction in permeabilized cells, suggesting that ET-1-induced contraction of esophagus depends on PTX-sensitive Gi3 protein. PKC inhibitor, H-7 or chelerythrine and protein tyrosine kinase (PTK) inhibitor, genistein inhibited ET-1 or C_2-induced contraction. These results suggest that these contractions are mediated by the activation of PKC and PTK pathway. PKC-ε antibody inhibited the contraction by ET-1 or C_2 . N-myristoylated peptide derived from the pseudosubstrate sequences of PKC, Myr-PKC-ε inhibited the contraction, suggesting that PKC-ε isozyme is involved in the contraction. PD98059, p44/p42 MAPK inhibitor, blocked the contraction induced by ET-1 or C_2 in a concentration-dependent manner, respectively. ET-1 or C_2 increased the intensity of the detection bands identified by phosphospecific p44/p42 MAPK antibody and PD98059 decreased the intensity of the bands as compared with ET-1 or C_2 stimulated cells respectively.

In conclusion, ET-1 or C_2 produced the contraction of circular muscle cells in cat esophagus. The contraction is mediated by ET-1 receptor coupled Gi3 protein, resulting in the activation of PKC-ε-protein and p44/p42 MAPK.

[OA-5] [04/20/2001 (Fri) 14:30 - 14:45 / Room 1]

Improved cytotoxicity of DA-125 through high-affinity DNA binding and potent inhibition of Topoisomerase II activity

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Purpose: (8s,10s)-8-(3-Aminopropanoyloxyacetyl)-10-[(2,6-dideoxy-2-fluoro-α-L-talopyra nosyl) oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacene-dione hydrochloride (DA-125) is a novel anthracycline derivative with anti-cancer activity. In the present study, we compared the cytotoxicity of DA-125 with that of doxorubicin in H4IIE rat hepatoma cells and investigated the mechanistic basis. Because activation of MAP kinases, in particular c-Jun N-terminal kinase (JNK), is implicated in apoptotic cell death, the signaling pathways responsible for DA-125-induced apoptosis were studied. Methods: Cytotoxicity and apoptosis were measured in H4IIE cells and cells stably transfected with a dominant negative mutant of JNK1 by MTT and TUNEL assays. Inhibition of topoisomerase II activity was determined in vitro. Drug accumulation and DNA binding affinity were monitored by fluorescence spectroscopy. Results: The extent of cytotoxicity by DA-125 was greater than that by doxorubicin (IC50, 11.5 vs. 70 μM). DA-125 induced apoptosis with 30-fold greater potency than that of doxorubicin. Inhibition of topoisomerase II by DA-125 was 4-fold greater The presence of excess β-alanine,a DA-125 moiety, failed to alter cytotoxicity and accumulation of DA-125, indicating that the improved cytotoxicity of DA-125 did not result from the β-alanine moiety. Greater cellular accumulation of DA-125 correlated with its high affinity DNA binding. Although neither PD98059 nor SB203580 altered the degree of cytotoxicity induced by DA-125, JNK1(-) stable cells exhibited ~2-fold greater viability than control cells. DA-125-induced apoptosis was also decreased

in JNK1(-)-transfected cells. Conclusions: DA-125 potently inhibited topoisomerase II activity and induced apoptosis with the high rate of prooxidant production. DA-125 exhibited high- affinity DNA binding with improved cellular drug accumulation. Apoptosis induced by DA-125 involved the pathway of JNK1, but not ERK1/2 or p38 kinase

[OA-6] [04/20/2001 (Fri) 14:45 - 15:00 / Room 1]

Altered Expression of Ferritin Light Chain (FLC) by Sulfur Amino Acid Deprivation in Hepa1c1c7 and Raw264.7 Cells: The Role of Cellular Ca2+ and Free Iron for Prooxidant Production

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Ferritin expression is induced by oxidative stress, which confers resistance to oxidative insults. Sulfur amino acid deprivation (SAAD) induces oxidative stress through a decrease in a GSH content. The molecular mechanisms of cell-type specific ferritin light chain (FLC) expression in association with increases in intracellular Ca2+ and free iron pools were investigated in Hepa1c1c7 and Raw264.7 cells exposed to SAAD. Intracellular Ca2+ level was rapidly increased by SAAD, followed by returning to control at later times. Sulfhydryl-containing compounds prevented the increase in intracellular Ca2+ by SAAD, supporting the role of redox-state in the regulation of Ca2+. Thapsigargin or Ca2+-free medium inhibited the increase in intracellular Ca2+, showing that Ca2+ originated from endoplasmic reticulum as well as from extracellular source. Inhibition of Ca2+ mobilization decreased fluorescence of free iron pool inside cells and inhibited dichlorofluorescein oxidation. Deferoxamine also inhibited dichlorofluorescein oxidation. Hence, the increase in cellular Ca2+ content coupled with elevation in intracellular free iron pool and subsequent prooxidant production. FLC protein level was detected by Western blotting in Raw264.7 cells, but not in Hepa1c1c7 cells. SAAD alone or in combination with FeSO4, however, down-regulated FLC expression. On the contrary, the FLC mRNA level was increased by SAAD in both Hepa1c1c7 and Raw264.7 cells. Calcium or iron chelators prevented increases in the FLC mRNA. These results provided evidence that oxidative stress by SAAD inhibited FLC protein expression but increased the mRNA level through intracellular Ca2+ and subsequent release of iron.

[OA-7] [04/20/2001 (Fri) 15:00 - 15:15 / Room 1]

DNA Adduct Formation, Induction of Apoptosis and Cell Cycle Arrest by N-Nitroso Metabolite of Carbofuran

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Carbofuran (CF) is one of the most widely used carbamate pesticide in the world applied to insect and nematode control. Due to its widespread use in agriculture and households, contamination of food, water, and air has become imminent, and consequently adverse health effects are inevitable in humans, animals, wildlife and fish. It has been reported that CF alone or in combination with other carbamate insecticides influences the level of reproductive and metabolic hormones such as thyroxine and corticosterone, and results in impairment of endocrine, immune and behavioral functions. In this study, we evaluated the effects of CF and its N-nitroso derivative N-nitrosocarbofuran (NOCF) on DNA adduct formation, genotoxicity, cell growth and apoptosis of CHL cells. NOCF, but not CF, induced the formation of O6- and N7-methylguanine DNA adducts in calf thymus DNA and induced genotoxicity determined by Ames test. NOCF inhibited the growth of Chinese hamster lung fibroblast