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.

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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