Apoptotic Mechanism Induced by Adenosine, and Prostaglandins In Tumor Cells

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Apoptosis, a physiological mode of cell death, is an endogenous cell suicide mechanism that can be selectively triggered in response to various stimuli.

In spite of recent recognition of adenosine, released by normal metabolic machinery in ischemic or hypoxic cells, as an apoptosis inducer in several types of normal and tumor cells, biological pathways through which adenosine participates in cell death program remains to be solved. We described here that apoptosis by adenosine is directly associated with reduction of c-Myc oncoprotein in human promyelocytic leukemia HL-60 cells. Before apoptotic features appeared, the expression of c-myc proto-oncogene was dramatically reduced from 30 minutes and was not recovered for six hours after the addition of 1 mM adenosine. The observations that both c-Myc inhibition and DNA fragmentation occurred by adenosine were efficiently blocked by 100 μM dipyridamole, a adenosine transport inhibitor, or s-(4-nitrobenzyl)-6-thioinosine(NBTI), a potent nucleosides uptake inhibitor not by adenosine receptor antagonists demonstrated an active transport system for Ado-induced apoptosis in these cells. Furthermore, we found that adenosine inserted into cells diminished intracellular GSH level and co-treatment with buthionine sulfoximine (BSO), an inhibitor of γ-glutamylcysteine synthetase, potentiated c-Myc reduction and apoptosis by adenosine. From our results, we suggested a possibility that adenosine, directly taken into HL-60 cells *via* an active transporter, decrease intracellular GSH production through blocking transmethylation cycle and leads ample c-Myc reduction for self destruction process.

Cyclopentenone prostaglandins(PGs) such as PGA_2 and Δ^{12}-PGJ_2 have potent antiproliferative activity on various cultured tumor cells. L1210 cells were treated with a cytotoxic dose of PGA_2 or Δ^{12}-PGJ_2 for 24h showed the characteristic morphological features and some cardinal biochemical findings of apoptosis. Human hepatocarcinoma cells(SK-HEP-1) were induced to die through apoptosis by treatment with Δ^{12}-PGJ_2 as characterized by appearance of a typical DNA ladder. The induction of apoptosis by Δ^{12}-PGJ_2 was specifically blocked by cycloheximide(CHX). Western analysis using anti-p53 or anti-WAF1 monoclonal antibodies demonstrated that these two protein levels were increased 3h after Δ^{12}-PGJ_2 treatment, and accumulation of p53 protein seemed to be dependent on the increase of p53 mRNA level, which was inhibited by CHX treatment. However, delayed addition of CHX after Δ^{12}-PGJ_2 treatment failed to affect both p53 mRNA level and DNA fragmentation following Δ^{12}-PGJ_2 treatment, indicate that the inhibition of p53 synthesis may contribute to the protective effect of CHX against Δ^{12}-PGJ_2 mediated cytotoxicity. These results suggested that the initial events caused by Δ^{12}-PGJ_2, leading ultimately to SK-HEP-1 cell death, invlove a certain process required for p53 induction. However, the finding that Δ^{12}-PGJ_2 is also active against Hep 3B cells which are devoid of a functional p53 indicates

that p53 may not be the critical requiremint for inducing apoptosis by Δ^{12}-PGJ_2. We studied the effect of intracellular glutathione(GSH), which was known to conjugate readily with an α, β unsaturated carbonyl of Δ^{12}-PGJ_2, on the apoptosis of Δ^{12}-PGJ_2. The Δ^{12}-PGJ_2 induced apoptosis was augmented by GSH depletion resulted from pretreatment with buthionine sulfoximine (BSO), an inhibitor of r-glutamylcysteine synthetase, on the contrary, N-acetyl-cysteine(NAC), a precursor of cysteine, elevated the GSH level and protected cells from initiating apoptosis by Δ^{12}-PGJ_2. These results suggest that the apoptosis inducing activity of Δ^{12}-PGJ_2 is due to thiol-reactivity and intracellular GSH modulates the Δ^{12}-PGJ_2 induced apoptosis by regulating the accessibility of Δ^{12}-PGJ_2 to target proteins containing thiol groups.

Human adenocarcinoma cells(SK-HEP-1) treated with PGA_2(10 μg/ml) resulted in a does-dependent increase in phosphorylation of a protein of approximate molecular mass of 17KDa. The phosphorylation of it may play a role in apoptosis though the exact identification and characterization remains to be elucidated.

Also, we performed differential display with mRNA that obtained from human hepatocellular carcinoma cells(Hep3B) treated with PGA_2(10 μg/ml). Differentially expressed bands, H1, H3 and S1, were recovered from the dried denaturing polyacrylamide gel. They were cloned into the pGEM3Z plasmid vector. Then their nucleotide seguence wes determined. In result, no genes with significant homology to H3 were found in the GenBank and EMBL DNA data bases.

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