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Prostaglandin D_2 and J_2 induce apoptosis in human leukemia cells via activation of the caspase 3 cascade and production of reactive oxygen species

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Abstract

The presence of prostaglandins (PGs) has been demonstrated in the processes of carcinogenesis and inflammation. In the present study, we found that 12-*o*-tetradecanoylphorbol 13-acetate (TPA) induced cyclooxygenase 2 (COX-2), but not COX-1, protein expression in HL-60 cells, and the addition of arachidonic acid (AA) in the presence or absence of TPA significantly reduced the viability of HL-60 cells, an effect that was blocked by adding the COX inhibitors, NS398 and aspirin. The AA metabolites, PGD_2 and PGJ_2, but not PGE_2 or $PGF_{2\alpha}$, reduced the viability of the human HL60 and Jurkat leukemia cells according to the MTT assay and LDH release assay. Apoptotic characteristics including DNA fragmentation, apoptotic bodies, and hypodiploid cells were observed in PGD_2- and PGJ_2-treated leukemia cells. A dose- and time-dependent induction of caspase 3 protein procession, and PARP and D4-GDI protein cleavage with activation of caspase 3, but not caspase 1, enzyme activity was detected in HL-60 cells treated with PGD_2 or PGJ_2. Additionally, DNA ladders induced by PGD_2 and PGJ_2 were significantly inhibited by the caspase 3 peptidyl inhibitor, Ac-DEVD-FMK, but not by the caspase 1 peptidyl inhibitor, Ac-YVAD-FMK, in accordance with the blocking of caspase 3, PARP, and D4-GDI protein procession. An increase in intracellular peroxide levels by PGD_2 and PGJ_2 was identified by the DCHF-DA assay, and anti-oxidant *N*-acetyl cysteine (NAC), mannitol (MAN), and tiron significantly inhibited cell death induced by PGD_2 and PGJ_2 by reducing reactive oxygen species (ROS) production. The PGJ_2 metabolites, 15-deoxy-$\Delta^{12,14}$-PGJ_2 and Δ^{12}-PGJ_2, exhibited effective apoptosis-inducing activity in HL-60 cells through ROS production via activation of the caspase 3 cascade. The proliferator-activated receptor-γ (PPAR-γ) agonists, rosiglitazone (RO), troglitazone (TR), and ciglitazone (CI), induced apoptosis in cells which was blocked by the addition of the PPAR-γ antagonists, GW9662 and BADGE, via blocking of caspase 3 and PARP cleavage. However, neither GW9662 nor BADGE showed any protective effect on PGD_2- and PGJ_2-induced apoptosis. A differential apoptotic effect of PGs through ROS production, followed by activation of the caspase 3 cascade, was demonstrated.

Keywords: Apoptosis; Caspase 3; ROS; Prostaglandin; Cyclooxygenase; PPAR-γ

1. Introduction

Prostaglandins (PGs) are a family of oxygenated metabolites of arachidonic acid (AA), and have a diverse range of actions depending on the PG type and cell target. PGs are divided into two groups, conventional PGs such as PGE_2, $PGF_{2\alpha}$, and PGD_2 and cyclopentenone PGs such as PGJ_2, PGA_1, and PGA_2 [1,2]. AA is the precursor of PGs, and is primarily converted to PGH_2 by

Abbreviations: MTT, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyMethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; TPA, 12-*o*-tetradecanoylphorbol 13-acetate; Ac-DEVD-FMK, acetyl-Asp-Glu-Val-Asp-fluoromethylketone; Ac-YVAD-FMK, acetyl-Tyr-Val-Ala-Asp-fluoromethylketone; DCHF-DA, dichlorodihydrofluorescein diacetate; PARP, poly (ADP-ribose) polymerase; NAC, *N*-acetyl-cysteine; ALL, allopurinol; DPI, diphenylene iodonium; ROS, reactive oxygen species; PGs, prostaglandins; BADGE, biphenol A diglycidyl ether; GW9662, 2-chloro-5-nitrobenzanilide

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cyclooxygenases followed by conversion of PGH_2 to several related PGs including PGD_2, PGJ_2, $PGF_{2\alpha}$, and PGE_2 by tissue-specific isomerase. Several physiological effects of PGs have been identified. PGE_2 production is increased in colon, gastric, and lung carcinomas with an increase in COX-2 protein levels [3,4]. Our previous data demonstrated that PGE_2 is involved in 12-*o*-tetradecanoylphorbol 13-acetate (TPA)- and epidermoid growth factor (EGF)-induced proliferation [5,6]. PGD_2 is a major product in a variety of tissues or cells, and has significant effects including platelet aggregation and vasorelaxation [7]. In vivo and in vitro studies have shown that PGD_2 readily undergoes dehydration to yield active PGs of the J_2 series including PGJ_2, Δ^{12-14} PGJ_2, and 15-deoxy-Δ^{12-14} PGJ_2 [8,9]. Members of PGJ_2 contain a reactive α,β-unsaturated ketone in the cyclopentenone ring that is important for their biological activities including anti-tumor, anti-inflammation, and antiviral replication effects [10–12].

Many types of mammalian cells undergo apoptosis during normal development or in response to a variety of stimuli, including DNA damage, oxidative stress, growth factor deprivation, and chemical treatment. Apoptosis induced by these agents appears to be regulated by a set of downstream genes such as p53, p21, caspases, and Bcl-2 family genes [13,14]. Human caspase-1 to -10 have been described, and previous studies demonstrated that activation of the caspase cascade is involved in chemical- and agent-induced apoptosis [15,16]. Caspase 3 exists as an inactive pro-caspase 3 in the cytoplasm and is proteolytically converted to active caspase 3 by a single cleavage event in cells undergoing apoptosis. After caspase 3 activation, some specific substrates for caspase 3 such as PARP and D4-GDI proteins are cleaved, and these are important for the occurrence of apoptosis [17,18].

Several previous studies suggested that PGs might reduce cell viability via apoptosis induction, but their mechanisms of action are complex and not well defined. Our previous study demonstrated that apoptosis induced by chemicals was mediated by activation of the caspase 3 cascade through a distinct ROS-dependent or -independent pathway [19,20]. In the present study, we obtained evidence that PGD_2 and PGJ_2, but not PGE_2 or $PGF_{2\alpha}$, exhibited effective apoptosis-inducing activities through ROS production and caspase 3 activation in human leukemia cells. ROS-dependent caspase 3 activation was identified in PGD_2- and PGJ_2-induced apoptosis.

2. Materials and methods

2.1. Cell culture

HL-60 and Jurkat human promyeloleukemic cells were obtained from ATCC (American Type Culture Collection; Rockville, MD). HL-60 and Jurkat cells were grown in RPMI1640 containing 10% heat-inactivated fetal bovine serum (FBS) and maintained at 37 °C in a humidified incubator containing 5% CO_2. Exponentially growing cells were exposed to drugs for the indicated time periods. All culture reagents were purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Chemicals

The colorigenic synthetic peptide substrates, Ac-DEVD-pNA, Ac-YVAD-pNA, Ac-DEVD-FMK, and Ac-YVAD-FMK, were purchased from Calbiochem. Propidium, iodide, PGs, TPA, and AA were obtained from Sigma Chemical (St. Louis, MO). Rosiglitazone (RO), troglitazone (TR), ciglitazone (CI), GW9662, and BADGE were obtained from Cayman Chemical. Antibodies for PARP, caspase 3, and D4-GDI detection in Western blotting were obtained from IMGENEX. Antibodies for detecting Bcl-2 family proteins and α-tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Dichlorodihydrofluorescein diacetate (DCHF-DA) was obtained from Molecular Probes.

2.3. Cell viability

Cell viability was assessed by MTT staining as described previously [21]. Briefly, cells were plated at a density of 10^5 cells/well in 24-well plates. After overnight growth, cells were treated under various conditions for 12 h. At the end of treatment, 30 μl of the tetrazolium compound, MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), and 270 μl of fresh RPMI medium were added. The supernatant was removed, and formazan crystals were dissolved in DMSO. After incubation for 4 h at 37 °C, 200 μl of 0.1 N HCl in 2-propanol was placed in each well to dissolve the tetrazolium crystals. At the end, the absorbance at a wavelength of 600 nm was recorded using an ELISA plate reader.

2.4. Determination of ROS production

ROS production was monitored by flow cytometry using DCFH-DA [19]. This dye is a stable compound that readily diffuses into cells and is hydrolyzed by intracellular esterase to yield DCFH, which is trapped within cells. Hydrogen peroxide or low-molecular-weight peroxides produced by cells oxidize DCFH to highly fluorescent compound, 2′,7′-dichlorofluorescein (DCF). Thus, the fluorescence intensity is proportional to the amount of peroxide produced by the cells. In the present study, HL-60 cells were treated with each of the indicated compounds for 2 h and washed twice with PBS to remove the extracellular compounds. DCHF-DA (100 μM) was added for an additional hour. Green fluorescence was excited using an argon laser and was detected using a 525-nm band-pass filter by flow cytometric analysis.