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Short communication

Bisdemethoxycurcumin enhances X-ray-induced apoptosis possibly through p53/Bcl-2 pathway

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ABSTRACT

Bisdemethoxycurcumin (BDMC), which is isolated from the rhizomes of *Curcuma longa,* has anti-inflammatory and anti-carcinogenic activities. Here we found that BDMC enhanced X-ray-induced apoptosis in human T-cell leukemia MOLT-4 cells. Knockdown of p53 significantly attenuated the radiosensitizing effect of BDMC. However, BDMC did not enhance X-ray-mediated activation of the p53 signaling pathway via p53's transactivation or mitochondrial translocation. On the other hand, BDMC promoted the X-ray-induced dephosphorylation at Ser 70 in Bcl-2's flexible loop regulatory domain and Bcl-2 binding to p53. Overexpressing Bcl-2 completely blocked the BDMC's radiosensitization effect. Our results indicate that BDMC stimulates the dephosphorylation and p53-binding activity of Bcl-2 and suggest that BDMC may induce a neutralization of Bcl-2's anti-apoptotic function, thereby enhancing X-ray-induced apoptosis.

1. Introduction

Curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC), collectively called curcuminoids, are major components of turmeric (*Curcuma longa*). Curcumin's pharmacological properties include anti-tumor and anti-inflammatory activities [1]. Curcumin inhibits growth in a variety of cancer cells, and can suppress tumorigenesis in vivo [2,3]. Curcumin induces apoptosis via multiple cell-signaling pathways; for example, it can activate p53-dependent apoptosis pathways or inhibit survival mechanisms such as the NF-κB pathway [2–4]. By inhibiting the activation of NF-κB, curcumin decreases the expression of NF-κB target genes including Bcl-2 and Bcl-$_X$L, which are anti-apoptotic factors [5–7]. Recent studies demonstrated that BDMC's activity is stronger than that of curcumin for many effects [8]. However, it remains largely unknown how BDMC exerts its biological effects.

Mitochondria are key cell-death regulators in the intrinsic apoptotic pathway, which responds to DNA damage. When mitochondria receive a death signal, the outer mitochondrial membrane (OMM) is permeabilized to allow the release of pro-apoptotic factors that induce the activation of caspases responsible for apoptosis [9]. This OMM permeabilization is regulated by the opposing actions of pro- and anti-apoptotic Bcl-2 family proteins [9,10]. For instance, Bcl-2 inhibits cell death by sequestering the pro-apoptotic proteins Bax and Bak at the OMM, thereby preventing the release of pro-apoptotic factors such as cytochrome *c*, AIF, and SMAC/DIABLO [11].

The human T-cell leukemia-cell line MOLT-4 is highly sensitive to ionizing radiation (IR), which causes it to undergo apoptosis [12,13]. We previously demonstrated that p53 is important for the radiation-induced apoptosis of MOLT-4 cells; overexpressing short hairpin (sh)-type p53 siRNA (small interfering RNA) rescues MOLT-4 cells from radiation-induced apoptosis [13]. DNA damage not only upregulates p53's transcriptional activity, but also causes a portion of the p53 to translocate to the cytoplasm/mitochondria, where it can interact with Bcl-2 on the OMM and neutralize its anti-apoptotic function, leading to mitochondrial dysfunction [14,15].

Abbreviations: BDMC, Bisdemethoxycurcumin; Bcl-2, B-cell CLL/lymphoma 2; OMM, outer mitochondrial membrane; IR, ionizing radiation; NF-κB, nuclear factor for κ–kinase gene in B cells; KD, knockdown; Fr, fraction; FLD, flexible-loop regulatory domain; IP, immunoprecipitation; WCL, whole-cell lysates.

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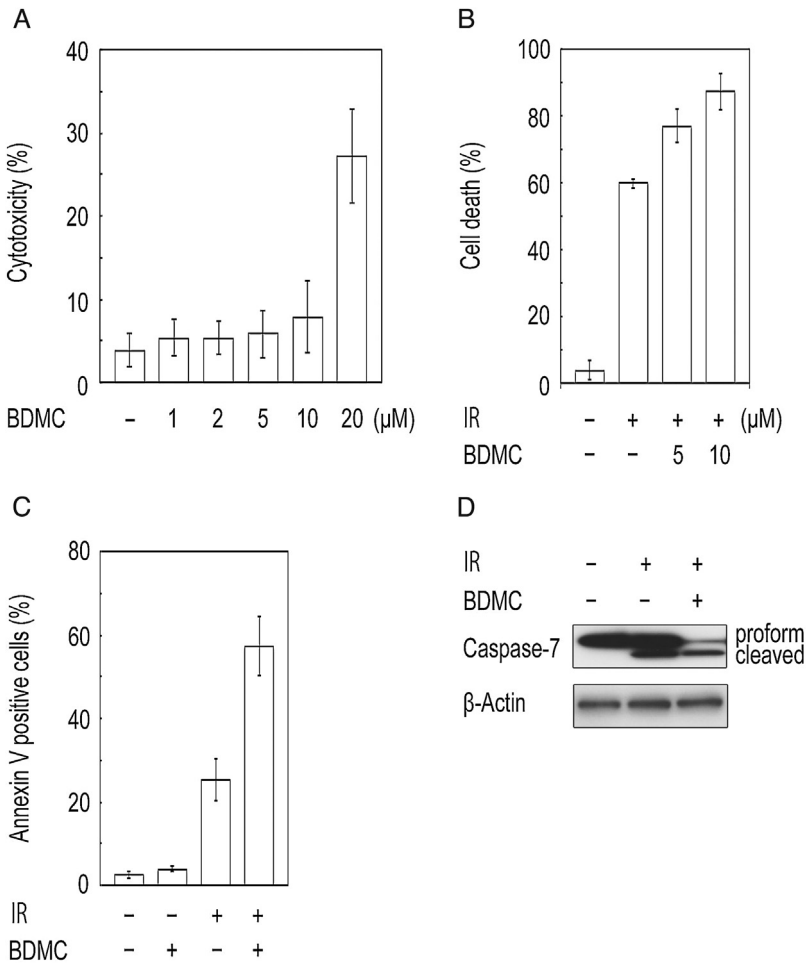

Fig. 1. BDMC enhances X-ray-induced apoptosis. (A) BDMC-induced cytotoxicity was examined by treating MOLT-4 cells with various concentrations of BDMC for 20 h and assessing cell death by staining with erythrosin B. (B) To determine BDMC's effect on X-ray-induced cell death, MOLT-4 cells were pretreated with DMSO or 5–10 μM BDMC for 1 h and then X-ray irradiated (2 Gy). Cell death was assessed 20 h after X-irradiation by staining with erythrosin B. (C) MOLT-4 cells were pretreated with DMSO or 10 μM BDMC for 1 h and X-ray irradiated (2 Gy). Apoptotic cell death was assessed 20 h after X-irradiation by staining with annexin V-FITC plus PI using a flow cytometer. Data in A-C are shown as means ± SD from 3 independent experiments. (D) The proteolytic cleavage of caspase- 7 during apoptosis was assessed by pretreating MOLT-4 cells with DMSO or 10 μM BDMC for 1 h and then subjecting them to X-ray irradiation (2 Gy). MOLT-4 cells were harvested 6 h after X-ray irradiation. Cell lysates were prepared and analyzed by western blot with the indicated antibodies; β-Actin was used as a loading control.

In this study, we demonstrate that BDMC promotes X-ray-induced apoptosis accompanied by increases in the dephosphorylation and p53 binding of Bcl-2 in MOLT-4 cells.

2. Material and methods

2.1. Cell culture and X-ray irradiation

Human T-cell leukemia MOLT-4 cells were grown in RPMI 1640 medium (Gibco, Grand Island NY) supplemented with 10% (v/v) fetal bovine serum (Hyclone, Longan, UT) and antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin). MOLT-4 stable transformants overexpressing sh-type p53 siRNA (MOLT/p53KD-1), negative-control shRNA (MOLT/Nega), or mouse bcl-2 (MOLT-4/mbcl-2) were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum and antibiotics in the presence of 0.5 mg/ml G418 (Sigma, St. Louis, MO) for maintenance. Logarithmically growing cells were pretreated with either DMSO or BDMC for 1 h and then irradiated in the medium with an X-ray generator (Pantak HF 350, Shimadzu, Kyoto) operating at 200 kV–20 mA with a filter of 0.5 mm Cu and 1 mm Al at a dosage rate of 1.33 Gy/min; 46 cm FSD. The unirradiated and irradiated cells were harvested at the indicated times without changing the medium.

2.2. Reagents

The curcumin derivative bisdemethoxycurcumin, purchased from Sigma Aldrich, was dissolved in DMSO.

2.3. Western blot analysis

Equivalent amounts of total cell lysates were separated by SDS-PAGE. Subcellular fractionation was performed with a ProteoExtract Subcellular Proteome Extraction Kit (Calbiochem, Darmstadt). Proteins separated in the gel were transblotted onto Immobilon PVDF membranes (Millipore, Bedford, MA). The membranes were blocked in Tris-buffered saline containing 0.05% Tween 20 (TBS-T) and 5% non-fat dry milk, and were incubated with anti-p53 (clone DO-1, Santa Cruz Biotechnology, Santa Cruz, CA), anti-Bcl-2 (N-19, Santa Cruz Biotechnology), anti-mouse-Bcl-2 (Upstate Biotechnology, Lake Placid, NY), anti-Bax (Biolegend, San Diego, CA), anti-phospho-p53 (Ser 15) (Cell Signaling Technology, Beverly, MA), anti-phospho-Bcl-2 (Ser 70) (Cell Signaling Technology), anti-Puma (Ab-1, Calbiochem), anti–Caspase- 7 (clone 4G2, MBL, Nagoya), anti-CDK2 (Santa Cruz Biotechnology), anti-α–Tubulin (Wako Pure Chemical Industries, Osaka), or anti-β-Actin (clone AC-15, Sigma) antibody. The blots were triple-washed with