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Original article

Curcumin inhibits Jurkat cell proliferation by inducing apoptosis via activation-induced cell death

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ABSTRACT

The effects of curcumin on cell proliferation, activation-induced cell death (AICD) and expression of interleukin-2 (IL-2) receptors (IL-2Rα, CD25) by Jurkat cells were investigated. Incubation of Jurkat cells for 72 h with either phytohaemagglutinin (PHA) or curcumin (2.5 μg/mL) resulted in marked inhibition of cell proliferation, determined by ^{3}H-thymidine incorporation. The anticancer activity of curcumin was also confirmed by quantitating the PHA-induced AICD in Jurkat cells by flow cytometry. It was found that curcumin (2.5 μg/mL) enhanced apoptosis in PHA-treated Jurkat cells. Further we observed the level of CD25 expression on Jurkat cells by flow cytometry. Our data indicate that curcumin inhibited PHA-induced CD25 expression. The present study suggests that curcumin have potent anticancer activities, which might be useful in lymphoma treatment.

1. Introduction

New cytotoxic agents are urgently needed for the treatment of cancer because of the poor long-term response of this disease to conventional chemotherapy. There is growing concern among health scientists that populations with greater reliance on fruits and vegetables in the diet experience a reduced risk for the major cancers [1,2]. The major classes of phytochemicals with disease-preventing functions are antioxidants, detoxifying agents and immunostimulatory agents. Such dietary phytochemicals include curcumin (diferuloylmethane), a major naturally-occurring phenolic compound obtained from the rhizome of the plant turmeric (*Curcuma longa)*, which is used as a spice or yellow coloring agent for foods or drugs [3,4]. Curcumin is a major active component of turmeric and it gives specific flavor and yellow color to curry. This phytochemical has long been known to have broad antioxidant properties [5]. Because curcumin can suppress cancer cell proliferation, induce apoptosis, inhibit angiogenesis, suppress the expression of anti-apoptotic proteins while protecting immune system of the tumor bearer, it may have untapped therapeutic value [3,6,7]. Nonetheless, curcumin, a plant-based product, has shown significant promise against cancer and other inflammatory diseases. In our previous study we have reported the effect of curcumin on mitogen (phytohaemagglutinin [PHA]) stimulated T cell proliferation, natural killer (NK) cell cytotoxicity, production of cytokines by human peripheral blood mononuclear cells (PBMCs), and nitric oxide (NO) production in mouse macrophage cells, RAW-264.7 and also the gel shift assay to elucidate the effect of curcumin on nuclear factor-κB (NF-κB) expression. We observed that curcumin inhibits PHA-induced T cell proliferation, interleukin-2 production, NO generation, and lipopolysachharide-induced NF-κB and augments NK cell cytotoxicity [8]. Curcumin (diferuloylmethane) has also been shown to block many reactions in which NF-κB plays a major role. The compound has been shown to display anticarcinogenic properties in animals as indicated by its ability to inhibit both tumor initiation induced by benz(α) pyrene and 7,12-dimethylbenz (α) anthracene [9–11] and tumor promotion induced by phorbol esters [12,13] which are known to activate NF-κB. Curcumin has also been shown to inhibit type 1 human immunodeficiency virus long terminal repeat (HIV-LTR) directed gene expression and virus replication stimulated by TNF and phorbol ester [14], which likewise require NF-κB activation. In the present report we show that curcumin is a potent inhibitor of cancer cell proliferation. The results also indicate that curcumin enhances activation-induced cell death in Jurkat cells stimulated with PHA. This study shows that curcumin is a potential candidate for T cell lymphoma treatment.

2. Material and Methods

Human T cell lymphoma cell line (Jurkat) was grown in RPMI-1640 (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Biological Industries, Kibbutz Beik Haemek,

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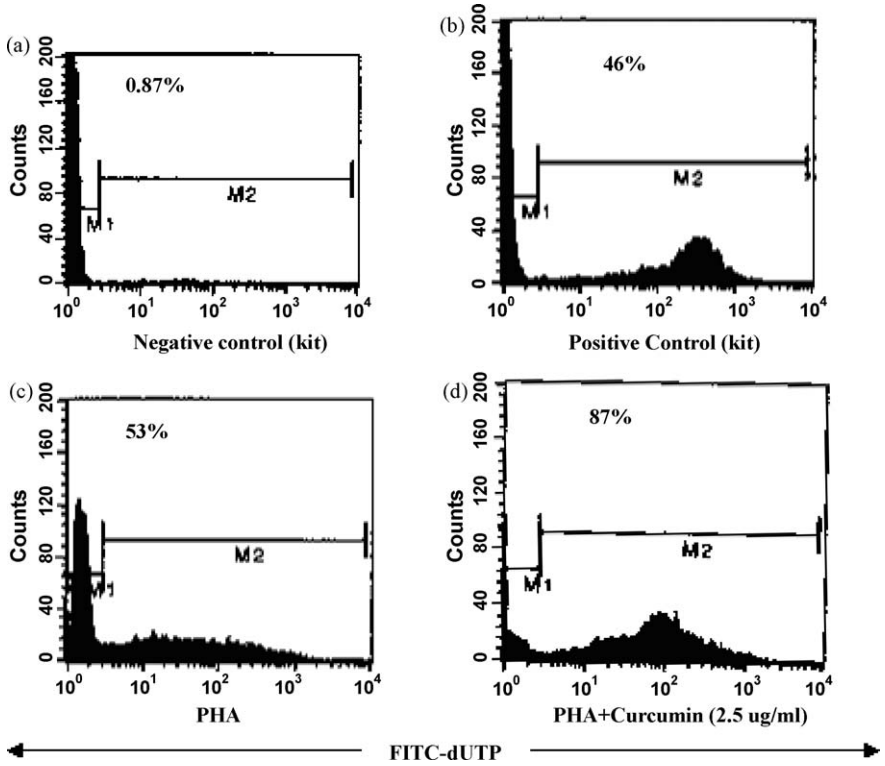

Fig. 1. Histograms showing apoptosis in Jurkat cells by Apo-Direct™ Kit (Beckton Dickinson, USA). Curcumin (2.5 ug/mL) increases apoptosis in PHA stimulated Jurkat Cells.

Israel), penicillin (100 U/mL) and streptomycin (100 μg/mL). Phytohaemagglutinin (PHA) was procured from Sigma. Apo-Direct™ kit was procured from BD-Pharmingen, USA.

2.1. Jurkat cell proliferation assay

The Jurkat cell proliferation test was carried out by [^{3}H] incorporation assay as described by Mishra et al. [15] with some modification. In brief, triplicate cultures were set up in 96 well round bottom plates, each well containing 0.2 mL of cell suspension (0.1×10^6 cells/mL) with or without PHA and curcumin or curcumin alone. The positive stimulant included PHA (5 μg/mL, Sigma). The cultures were incubated for 72 h and were pulsed with 0.5 mCi [^{3}H]-thymidine (6 Ci/mmol, BARC, Bombay, India) per well during the last 18 h of incubation. Thymidine uptake was determined by liquid scintillation counter (LKB, Fullerton, CA, USA). The mean count per minute (CPM) of the triplicate cultures was calculated for each set. Data have been presented as CPM ± SD (standard deviation).

2.2. Quantitation of apoptosis by Apo-Direct™ kit

Apoptosis was measured by Apo-Direct™ kit as per manufacturer's instruction briefly 2×10^6 cells suspended in cultured overnight with PHA (5 μg/mL) and curcumin (2.5 μg/mL) in 0.5 mL of PBS and added the cell suspension into 5 mL of 1% (W/V) paraformaldehyde in PBS and placed on ice for 15 minutes. Cells were centrifuged for 5 minutes at 300 g and supernatant were discarded. Further cells were washed in 5 mL of PBS then pelleted the cells by centrifugation and repeated the wash and centrifugation steps. Cells were resuspended in 0.5 mL of PBS and further added to 5 mL of ice-cold 70% ethanol. Staining was performed as per manufacturer's instruction. Apoptosis was assessed by terminal deoxynucleotidyltransferase-mediated dUTP end-labeling staining using the Apo-Direct™ kit (PharMingen). Expected results using the positive and negative control cells were shown in Fig. 1b and c.

2.3. Staining for cell surface markers

Jurkat cells were incubated overnight with curcumin either alone or in combination with PHA. Cell surface expression of CD25 was evaluated by FITC conjugated monoclonal antibody by flow cytometry. Analysis was performed by flow cytometry [16]. Briefly, Jurkat cells were incubated with curcumin overnight. Further, it was incubated with saturating amounts of PE/FITC-coupled antibody at room temperature in the dark for 30 minutes. Cells were then washed twice in PBS (pH 7.2) and finally resuspended in 500 μL of sheath fluid (Becton Dickinson, CA, USA). Background fluorescence was assessed with the appropriate isotype and fluorochrome-matched control monoclonal antibody. Analysis was carried out in FACScan using Cell Quest software. PHA was used as a positive stimulant for CD25.

3. Results

3.1. Effect of curcumin on Jurkat cell proliferation

Jurkat cells, a T cell lymphoma of human origin, were treated with or without PHA (5 μg/mL) and different concentrations of curcumin. PHA treatment lead to activation-induced cell death (AICD) in Jurkat cells. Lower concentrations of curcumin inhibited AICD. However, higher concentration (2.5 μg/mL) of curcumin induced AICD (Table 1).

3.2. Effect of curcumin on activation-induced cell death

Jurkat cells 2×10^6 were treated with (or without) PHA and curcumin overnight, fixed and washed. Stained 1×10^6 cells in the staining solution for 30 minutes at 37 °C. Cells were analysed to see the effect of curcumin (2.5 μg/mL) on activation-induced cell death. In the PHA-treated Jurkat cell, activation-induced cell death (AICD) was 57% while curcumin enhanced the AICD to 87%. Data suggest