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PKC inhibition is involved in trichosanthin-induced apoptosis in human chronic myeloid leukemia cell line K562

Jie Li [a], Xuechun Xia [a], Huiling Nie [a,*], Mark A. Smith [b], Xiongwei Zhu [b,*]

[a] *Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Science, Graduate School of the Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, China*
[b] *Department of Pathology, Case Western Reserve University, 2103 Cornell Road, Cleveland, OH 44106, USA*

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Abstract

Trichosanthin (TCS), a type I ribosome-inactivating protein, induces cell death in various cell types including several tumor cell lines. However, the mechanism remains largely uncharacterized. In this study, we investigated the possible mechanism underlying its cytotoxicity by using human chronic myeloid leukemia cell line K562. We found that TCS induced apoptosis in K562 cells in a time- and concentration-dependent manner and can be blocked by caspase-3 inhibitors. Interestingly, TCS treatment induced a transient elevation in intracellular calcium concentration and a slow increase in reactive oxygen species production, while calcium chelators and antioxidants had no obvious effect on TCS-induced apoptosis, suggesting that calcium changes and reactive oxygen species may not be involved in TCS-mediated apoptosis in K562 cells. Instead we found that TCS partly inhibited PKC activity. Indeed, the PKC activator, PMA, inhibited while the PKC inhibitor, calphostin c, enhanced TCS-induced apoptosis. These PKC modulators had similar effects on TCS-induced cleavage of caspase-3, and caspase-3 inhibitors prevented calphostin c-enhanced apoptosis induced by TCS. In summary, we conclude that TCS induces apoptosis in K562 cells partly via PKC inhibition and caspase-3 activation.

Keywords: Trichosanthin; Apoptosis; Calcium; PKC; Caspase-3

1. Introduction

Trichosanthin is a 27 kDa protein isolated from the root tuber of a traditional Chinese medicinal herb *Trichosanthes kirilowii* that has long been used in China to terminate early and midtrimester pregnancies and reset menstruation [1]. It is a well-characterized type I single chain ribosome-inactivating protein (RIP) that inactivates eukaryotic ribosomes through its RNA N-glycosidase activity [2,3]. TCS has been found to have broad pharmacological activities including abortifacient, anti-tumor, anti-HIV, and immunoregulatory which seem to associate with its ability to induce apoptosis in various cell types including OVA-specific T cell, HIV-infected H9 cells and different tumor cells (i.e., human choriocarcinoma cells, HeLa cells and murine melanoma cells) [4–8]. However, the mechanism of TCS-induced apoptosis remains elusive.

Apoptosis is the physiological process of cell suicide that occurs normally during development or as a stress response and it is widely accepted that apoptosis is an essential cellular response to agents used for anticancer chemotherapy and radiotherapy [9–11]. Central to this suicide program is a cascade of caspases with caspase-3 being one of the key executioners which in turn is cleaved/activated by initiator caspases [12]. In mammalian cells, the caspase cascade can be activated by two pathways, i.e., the intrinsic and extrinsic cell death-inducing pathways, which are regulated by various

Abbreviations: cal c, calphostin c; DEVD-CHO, N-acetyl–Asp–Glu–Val–Asp aldehyde; H_2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; NAC, N-acetyl cysteine; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PS, phospholipidserine; RIP, ribosome-inactivating protein; ROS, reactive oxygen species; TCS, trichosanthin; Z-VAD-FMK, benzyloxycarbonyl–Val–Ala–Asp–fluoromethylketone

* Corresponding authors. H. Nie is to be contacted at fax: +86 21 54921384. X. Zhu, Department of Pathology, Case Western Reserve University, 2103 Cornell Road, Cleveland, Ohio 44106, USA.
E-mail addresses: hlnie@sibs.ac.cn (H. Nie), Xiongwei.Zhu@case.edu (X. Zhu).

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upstream signaling events [12]. Among these cellular signaling events that lead to apoptosis, $[Ca^{2+}]_i$ elevation and protein kinase C (PKC) activation play critical roles [13–17].

In this study we investigated the mechanism involved in the TCS-induced cell death in human erythroleukemia cells K562. We demonstrated that TCS induced apoptosis in K562 cells in a time- and dose-dependent manner which involved caspase-3 cleavage. Although a rapid and transient calcium elevation was induced by TCS in K562 cells, it seemed to not be involved in TCS-induced apoptosis. Instead, we found that PKC activity was partly inhibited by TCS treatment which underlies TCS-induced apoptosis.

2. Materials and methods

2.1. Materials

TCS was purchased from Shanghai Jinshan Medical Factory. NAC, Trolox, PMA, EGTA, BAPTA/AM, poly-L-lysine and anti-actin polyclonal antibody were purchased from Sigma. Calphostin c, Ac-DEVD-CHO and Z-VAD-FMK were obtained from Calbiochem. PKC activity assay kit was purchased from Upstate. Anti-caspase-3 polyclonal antibody was purchased from Cell Signaling Technology. Annexin V-FITC was purchased from Oncogene. RPMI 1640 medium and fetal bovine serum were purchased from Invitrogen. BCA protein assay kit was from PIERCE.

2.2. Cell culture

The human chronic myeloid leukemia cell line K562 was obtained from ATCC (Rockvillie, MD) and maintained in complete RPMI 1640 medium containing 10% (v/v) heat-shocked fetal serum and 100 μg/ml penicillin, 100 μg/ml streptomycin. The culture was incubated at 37 °C in a humidified 5% CO_2/95% air.

2.3. Flow cytometry

At different time points, treated cells and controls were washed and adjusted to 1×10^6 /ml in fixation. After washing, the $200\times g$ centrifuged cell pellets were resuspended in 1 ml of hypotonic fluorochrome solution [propridium iodide (PI) 50 μg/ml in 0.1 M sodium citrate plus 0.1% Triton X-100; Sigma]. The samples were placed overnight in the dark at 4 °C, and the PI fluorescence of individual nuclei measured using a FACScan flow cytometer as described by [18]. Data were evaluated using Modfit (DNA Modeling System) software and expressed as fractions of cells in the different cycle phases. The experimental values represented the mean from three independent experiments.

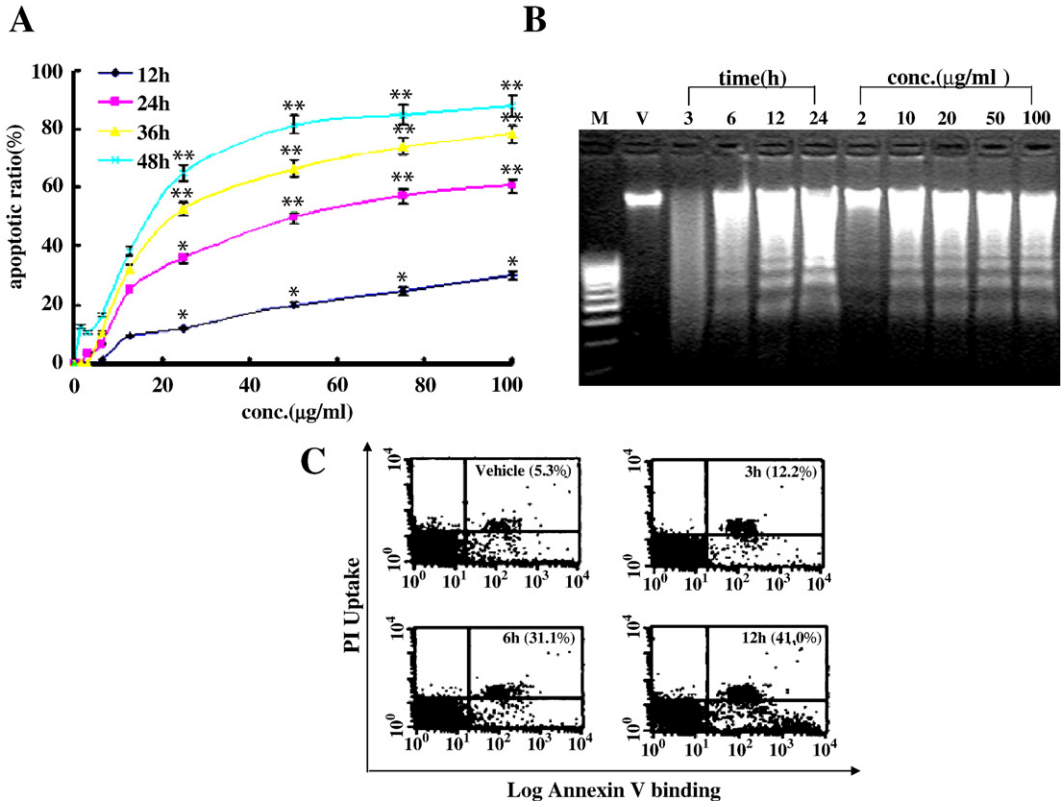

Fig. 1. TCS induced apoptosis in K562 cells in a dose- and time-dependent manner. (A) K562 cells were treated with a range of concentrations (0–100 μg/ml) of TCS over different time periods and cell apoptosis was determined by flow cytometry. The percentage of apoptotic cells was calculated as a ratio of sub-G0/G1 (the sub-diploid peak) in total cells. Values are shown as mean±SEM of three independent experiments. *$P<0.05$, **$P<0.01$, compared with untreated cells. (B) TCS induced DNA fragmentation in K562 cells. After the cells were treated with TCS at 100 μg/ml for 3, 6, 12, 24 h, respectively, or at various concentration for 24 h, the cellular DNA was extracted, dialyzed and separated on a 1.8% agarose gel and visualized under UV light by EB staining (three independent experiments). (C) Contour diagram of annexin V-FITC/PI flow cytometry of K562 cells treated for the indicated time intervals with and without (control) 100 μg/ml TCS. The lower left quadrant of each panel shows the viable cells, which exclude PI and are negative for Annexin V-FITC binding. The upper right quadrants contain the non-viable, necrotic cells, positive for both annexin V-FITC binding and PI uptake. The lower right quadrants represent the apoptotic cells, annexin V-FITC positive and PI negative, demonstrating cytoplasmic membrane integrity. Ten thousand cells were analyzed at each time point. One representative experiment out of three independent experiments is shown. Number in parenthesis represents the percentile of apoptotic cells (lower right quadrants).