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Cucurbitacin B induces differentiation, cell cycle arrest, and actin cytoskeletal alterations in myeloid leukemia cells

Talin Haritunians [a,*,1], Saskia Gueller [a,1], Ling Zhang [b], Riem Badr [b], Dong Yin [a], Hongtao Xing [a], Ming Chiu Fung [c], H. Phillip Koeffler [a,2]

[a] *Division of Hematology/Oncology, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA, USA*
[b] *Division of Pathology, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA, USA*
[c] *Department of Biology, Chinese University of Hong Kong, Hong Kong, China*

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Abstract

Cucurbitacins have long been utilized for their abortifacient and anti-inflammatory effects; however, little is known about their mechanism of action. In this study, we have demonstrated robust antiproliferative effects of CuB on various leukemia and lymphoma cell lines, as well as on primary mononuclear bone marrow cells derived from patients with acute myeloid leukemia or myelodysplastic syndrome. Myeloid leukemic cells treated with CuB exhibit significant S-phase cell cycle arrest, enlarged cell size, multinucleation, and enhanced expression of a monocytic- and granulocytic-specific CD11b. Moreover, our data demonstrate that CuB prominently alters the cytoskeletal network of leukemic cells, inducing rapid and improper polymerization of the F-actin network. These encouraging results suggest the appropriateness of clinical trials of cucurbitacins for the treatment of hematopoietic malignancies.

Keywords: Cucurbitacin; Leukemia; Myelodysplastic syndrome; Actin cytoskeleton

1. Introduction

Naturally occurring cucurbitacins are cytotoxic terpene sterols containing a cucurbitane skeleton characterized by a 19-(10 → 9β)-abeo-10α-lanost-5-ene. Present in numerous plant families, cucurbitacins A–T are chemically very diverse [1]. Several different cucurbitacin compounds have been isolated and have been found to exhibit antiproliferative and cytotoxic activity both in vitro and in vivo. The effectiveness of cucurbitacins B, D, E, and I, has so far been shown in colon (HCT-116), breast (MCF-7), lung (NCI-H460) and brain (SF-268) cancer cell lines, in which cucurbitacin B demonstrated more than 80% inhibitory effect on proliferation [2]. Another study revealed growth inhibition accompanied by cell cycle arrest and apoptosis in breast cancer cell lines (MCF-7 and MDA-MB-231) upon treatment with cucurbitacins B and E [3]. Choriocarcinoma cells revealed sensitivity towards treatment with cucurbitacins as well [4]. Cucurbitacins significantly inhibited tumor growth in a nude mouse xenograft model using A549 lung cancer, v-Src transformed NIH3T3 or MDA-MB-468 breast cancer cells [5,6]. Cucurbitacins also inhibited proliferation of normal mitogen-induced T-lymphocytes [7] and endothelial cells accompanied by a disruption of the F-actin cytoskeleton and reduced cell motility [8]. The latter effects suggest a role of cucurbitacins in anti-angiogenesis and anti-metastasis. Little is known about the mechanism of action of cucurbitacins, and several opposing effects on cellular signalling have been reported. On the one hand, cucurbitacins A, B, E, I and Q inhibited phosphorylation of STAT3 and/or Jak2 in lung cancer cells (A549); likewise, cucurbitacin I caused reduction of phospho-STAT3 in breast and prostate carci-

* Corresponding author at: Division of Hematology/Oncology, Cedars-Sinai Medical Center, Davis 4094B, 8700 Beverly Blvd., Los Angeles, CA 90048, USA. Tel.: +1 310 423 3295; fax: +1 310 423 0443.
E-mail address: haritunianst@cshs.org (T. Haritunians).
[1] These authors contributed equally to this work.
[2] H. Phillip Koeffler holds the Mark Goodson Chair of Oncology Research at Cedars-Sinai Medical Center and is a member of the Molecular Biology Institute and Jonsson Comprehensive Cancer Center at UCLA.

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Table 1
Cucurbitacins B and D inhibit growth of various hematopoietic leukemia and lymphoma cell lines

Cell lines		Inhibition of proliferation, ED50 (M)	
		CuB	CuD
HL60	Acute promyelocytic leukemia	4.0×10^{-8}	2.0×10^{-7}
U937	Acute myeloid leukemia	2.5×10^{-8}	2.0×10^{-7}
THP1	Acute monocytic leukemia	1.5×10^{-8}	8.0×10^{-8}
NB4	Acute promyelocytic leukemia	2.0×10^{-8}	–
K562	Chronic myeloid leukemia	1.3×10^{-8}	–
BALL1	B-cell acute lymphocytic leukemia	4.0×10^{-8}	2.0×10^{-7}
Reh	B-cell acute lymphocytic leukemia	2.0×10^{-8}	9.0×10^{-8}
RCH	B-cell acute lymphocytic leukemia	7.0×10^{-9}	4.0×10^{-8}
Daudi	Burkitt's lymphoma	2.0×10^{-8}	9.0×10^{-8}
LY4	Diffuse large B-cell lymphoma	6.5×10^{-8}	9.5×10^{-8}
MD901	Diffuse large B-cell lymphoma	4.0×10^{-8}	8.0×10^{-8}
SP49	Mantle cell lymphoma	5.5×10^{-8}	1.5×10^{-7}
Jeko1	Mantle cell lymphoma	5.0×10^{-8}	9.0×10^{-8}
NCEB1	Mantle cell lymphoma	6.0×10^{-8}	1.0×10^{-7}

Cells were cultured for 96 h with varying doses of either CuB or CuD and cell growth was analyzed by MTT assay. Data were plotted, and the effective dose which inhibited 50% growth (ED50) was calculated for each cell line.

noma cell lines (MDA-MB-231, MDA-MB-468, Panc-1) [5,6]. Also cucurbitacin I had greater anticancer activity in nude mice xenograft models using cell lines with activated STAT3 compared to those that are independent of STAT3 [5]. Surprisingly, cucurbitacins B and E have been shown to induce phosphorylation of STAT3 in breast cancer cell lines (MDA-MB-231, MCF-7) while still exhibiting growth inhibition [3]. Furthermore in HeLa cells, cucurbitacins inhibited DNA, RNA, and protein synthesis [9]. Selective cucurbitacins have been reported selectively to inhibit COX2 activity [5,10] and alter cell morphology by disruption of the F-actin cytoskeleton [8,11,12]. Thus, different cucurbitacin compounds can have heterogenous effects that also depend on the target cells. Therefore, the aim of our study was to elucidate the effects of cucurbitacin on hematopoietic cells. To date, scant data describing cucurbitacin effectiveness in hematopoietic cells are available. The only study found that cucurbitacin I inhibited proliferation and induced apoptosis of two anaplastic large cell lymphoma cell lines [13]. Therefore, our aim was to evaluate the effects of CuB on various hematopoietic cell lines and on bone marrow cells isolated from patients with either acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). We used chemically purified cucurbitacin B (CuB) and cucurbitacin D (CuD), which were extracted from *Trichosanthes kirilowii* Maximowicz (Cucurbitaceae family). The fruit and roots of this plant have long been utilized in oriental medicine for their anti-inflammatory and abortifacient effects. Our data show that CuB possessed strong antiproliferative effects, induced significant S-phase arrest and enhanced expression of CD11b, a marker of differentiation. Furthermore, CuB caused the leukemic cells to enlarge and form multinucleated cells. The agent rapidly altered the cytoskeletal integrity of the cells, causing improper filamentous (F)-actin polymerization.

2. Materials and methods

2.1. Cell culture

B-cell acute lymphocytic leukemia (RCH, kindly provided by Dr. Janet Rowley, University of Chicago; Reh, a generous gift from Dr. Gary Gilliland, Harvard University; BALL-1, kindly provided by Dr. Sven de Vos, University of California, Los Angeles), Burkitt's lymphoma (Daudi), diffuse large B-cell lymphoma (MD901, kindly provided by Dr. Miki, Tokyo Medical and Dental University, Japan; LY4, generously donated by Ari Melnick, Albert Einstein College of Medicine), and myeloid leukemia cell lines (HL60, U937, THP1, K562, and NB4, the latter a gift from Dr. Lanotte, INSERM, Hospital Saint-Louis, France) were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA), 10 U/ml penicillin and 10 mg/ml streptomycin (P/S) (Invitrogen) at 37 °C in 5% CO_2. Mantle cell lymphoma cell lines (NCEB1, Jeko1, and SP49) [14] were maintained under similar conditions in RPMI 1640 supplemented with 20% FBS and P/S. Unless otherwise noted, cell lines were purchased from American Type Culture Collection (Manassas, VA). Table 1 gives an overview of the cell lines used in this study.

2.2. MTT proliferation assays

Approximately 10^4 cells were plated per well in a 96-well plate and treated with either dimethyl sulfoxide (DMSO, diluent control) or various concentrations (1×10^{-9} to 1×10^{-6} M) of either CuB or CuD (kindly provided by CK Life Sciences Int'l., Inc., Hong Kong) for 96 h, a time point selected as it was previously determined to be optimal for MTT assays [15]. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma–Aldrich, St. Louis, MO) was performed as previously described [15]. In brief, 10 μl of MTT dissolved in phosphate-buffered saline (PBS) at 5 mg/ml was added to each well and incubated for 4 h. Then 100 μl of solubilization solution (20% sodium dodecyl sulfate [SDS]) was added, and the mixture was incubated again at 37 °C for 16 h. In this