Phytomedicine 23 (2016) 641–653

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Induction of apoptosis in human leukemia cells through an intrinsic pathway by cathachunine, a unique alkaloid isolated from *Catharanthus roseus*

Xiao-Dong Wang[a,c,d,1], Chen-Yang Li[a,c,d,1], Miao-Miao Jiang[b], Dong Li[b], Ping Wen[e], Xun Song[a,c,d], Jun-Da Chen[a], Li-Xuan Guo[a], Xiao-Peng Hu[a,c,d], Guo-Qiang Li[f], Jian Zhang[a,c,d], Chun-Hua Wang[b,*], Zhen-Dan He[a,c,d,**]

[a] *Department of Pharmacy, School of Medicine, Shenzhen University, Shenzhen 518060, Guangdong, PR China*
[b] *Tianjin Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, PR China*
[c] *Institute of Biotherapy, Shenzhen University, Shenzhen 518060, Guangdong, PR China*
[d] *Engineering Laboratory of Shenzhen Natural Micromolecule Innovative Drugs, Shenzhen University, Shenzhen 518060, Guangdong, PR China*
[e] *Business Technology Department, Shenzhen Institute for Drug Control, Shenzhen 518057, Guangdong, PR China*
[f] *Experiment and Technology Center, Jinan University, Guangzhou 510632, Guangdong, PR China*

ARTICLE INFO

Article history:
Received 26 May 2015
Revised 1 March 2016
Accepted 4 March 2016

Keywords:
Cathachunine
Apoptosis
Intrinsic pathway
ROS

ABSTRACT

Background: *Catharanthus roseus* (L.) G. Don consists of a range of dimeric indole alkaloids with significant antitumor activities. These alkaloids have been found to possess apoptosis-inducing activity against tumor cells *in vitro* and *in vivo* mediated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and c-Jun *N*-terminal kinase (JNK) pathways, in which DNA damage and mitochondrial dysfunction play important roles. In this study, a unique bisindole alkaloid named cathachunine, along with five known dimeric indole alkaloids, was obtained from *C. roseus* and investigated *in vitro*.
Purpose: The aim of this study was to investigate the antitumor activity of isolated alkaloids and the mechanism through which cathachunine exerts its antitumor effect.
Study design and methods: Cell growth inhibition was assessed by WST-1 and lactate dehydrogenase (LDH) assays in HL60, K562 leukemia cells and EA.hy926 umbilical vein cells. Induction of apoptosis in HL60 cells was confirmed by observation of nuclear morphology, a caspase-3 activity assay and annexin V-fluorescein isothiocyanate/propidium iodide (FITC/PI) double staining. The intrinsic apoptotic pathway induced by cathachunine was evidenced by B-cell lymphoma 2/Bcl-2-associated X protein (Bcl-2/Bax) dysregulation, loss of mitochondrial membrane potential, translocation of cytochrome *c*, and cleavage of caspase-3 and poly-ADP ribose polymerase (PARP). Reactive oxygen species (ROS) production after cathachunine treatment was determined by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) staining. Cell cycle arrest of the S phase was also observed in HL60 cells after cathachunine treatment.
Results: The WST-1 and LDH assays showed that *Catharanthus* alkaloids were cytotoxic toward human leukemia cells to a greater extent than toward normal human endothelial cells, and the anti-proliferation and pro-apoptosis abilities of cathachunine were much more potent than other previously reported alkaloids. The induction of apoptosis by cathachunine occurred through an ROS-dependent mitochondria-mediated intrinsic pathway rather than an extrinsic pathway, and was regulated by the Bcl-2 protein family.
Conclusion: An unprecedented bisindole alkaloid cathachunine which lost C-18′ and C-19′ was isolated from *C. roseus*. It exerted a potent antitumor effect toward human leukemia cells through the induction of apoptosis via an intrinsic pathway. Thus, this study provides evidence for a new lead compound from a natural source for anti-cancer investigations.

Abbreviations: ATCC, American type culture collection; DCFH-DA, 2′, 7′-dichlorodihydrofluorescein diacetate; DEPC, diethylpyrocarbonate; HL60, human promyelocytic leukemia cells; LDH, lactate dehydrogenase; MMP, mitochondrial membrane potential; OMM, outer mitochondrial membrane; PARP, poly-ADP ribose polymerase; PI, propidium iodide; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; RP-HPLC, reverse phase high performance liquid chromatography.

* Corresponding author. Tel./fax: +86 22 27386453.

http://dx.doi.org/10.1016/j.phymed.2016.03.003
0944-7113/

** Corresponding author at: Department of Pharmacy, School of Medicine, Shenzhen University, Shenzhen 518060, Guangdong, PR China. Tel.: +86 755 86671916; fax: +86 755 86671906.
E-mail addresses: pharmwch@126.com (C.-H. Wang), hezhendan@126.com (Z.-D. He).

[1] These authors contributed equally to this work.

Introduction

Catharanthus roseus (L.) G. Don is a pantropical plant widely studied by pharmacognosists that contains about 130 terpenoid indole alkaloids (van Der Heijden et al. 2004). Extracts from this plant have been used to treat numerous diseases, including diabetes, malaria and cancer. The alkaloids have been noted to exert an apoptosis-inducing ability towards tumor cells *in vitro* and *in vivo* mediated by the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and c-Jun *N*-terminal kinase (JNK) pathways, in which DNA damage and mitochondrial dysfunction play important roles (Chiu et al. 2012; Huang et al. 2004, 2012). Among the dimeric indole alkaloids with significant antitumor activity, vinblastine and vincristine are typical compounds that have been used extensively for the clinical treatment of human cancers, such as testicular carcinoma, acute leukemia, rhabdomyosarcoma and breast cancer. Vinblastine induces apoptosis in melanoma cells through mitochondrial and non-mitochondrial pathways mediated by the Rho A protein (Selimovic et al. 2013). Meanwhile, vincristine induces neuroblastoma cell death through mitotic arrest and mitochondria-dependent apoptosis (Tu et al. 2013). Previous investigations, including our own work, have attempted to identify diversiform dimeric indole alkaloids. These efforts have led to the identification of new alkaloids with significant cytotoxicity to human hepatocellular carcinoma (HepG2), human colorectal carcinoma (Lovo) and human breast carcinoma (MCF-7) cell lines (Wang et al. 2012, 2014; Zhang et al. 2013a, 2013b). However, further investigation is necessary for the chemical isolation of such alkaloids, as well as biological studies in order to understand more about their antitumor mechanisms.

Apoptosis is a highly regulated process of programmed cell death that can be triggered through either extrinsic or intrinsic pathways (Bai and Wang 2014). The extrinsic (death receptor) pathway begins with stimulation of death receptors on the cell membrane (Wallach et al. 2008). The intrinsic (mitochondrial) pathway is initiated by dysfunction of the mitochondria, which leads to the release of signaling factors, such as cytochrome *c* (cyto *c*), to the cytosol. Permeabilization of the mitochondrial membrane relies on the B-cell lymphoma 2 (Bcl-2) protein family, where pro-apoptotic Bcl-2-associated X (Bax) or Bcl-2 homologous killer (Bak) proteins oligomerize to form pores on the outer mitochondrial membrane (OMM; Tait and Green 2010). These two pathways are executed mainly by caspases (a family of cysteine proteases), with caspases-8 and -9 engaging in the extrinsic and intrinsic pathways, respectively (Ola et al. 2011). In addition, reactive oxygen species (ROS), a series of oxygen metabolism byproducts, have been closely related to cancer cell apoptosis induced by natural alkaloids (Kardeh et al. 2014).

In this study, a unique bisindole alkaloid named cathachunine (Fig. 1, **F**) was obtained from *C. roseus* and its chemical structure was elucidated by high resolution electrospray ionization mass spectrometry (HR-ESI-MS), 1D and 2D nuclear magnetic resonance (NMR), circular dichroism (CD) spectrophotometry, and infrared (IR) and ultraviolet (UV) spectroscopy. Subsequently, the cytotoxic mechanisms of cathachunine towards human leukemia cells were investigated, along with five other previously isolated compounds (leurosine, **A**; catharine, **B**; cycloleurosine, **C**; 15′-*R*-hydroxyvinamidine, **D**; and 17-deacetoxyvinamidine, **E**; Fig. 1). Growth inhibition in HL60, K562 leukemia and EA.hy926 umbilical vein cells by *Catharanthus* alkaloids was detected by WST-1 and lactate dehydrogenase (LDH) assays. The influence of the alkaloids on cell apoptosis was demonstrated through nuclear morphology observation, annexin V-fluorescein isothiocyanate/propidium iodide (FITC/PI) double staining and caspase-3 activity. Additional experiments were performed to investigate the apoptosis mechanism, which was found to be an ROS-dependent mitochondria-mediated intrinsic pathway. Cell cycle arrest of the S phase was also found in HL60 cells after treatment with **F**.

Materials and methods

General

IR and UV spectra were obtained on JASCO FT/IR-480 Plus and JASCO V-550 UV/VIS spectrophotometers, respectively. Melting points (mp) were recorded on an X-5 micro mp apparatus (uncorrected). NMR spectra were run on a Bruker AV-400 spectrometer. HR-ESI-MS data were detected on an Agilent 6210 ESI/TOF mass spectrometer. CD spectra were obtained on a JASCO J-810 spectropolarimeter at room temperature. High performance liquid chromatography (HPLC) separations were performed on a COSMOSIL

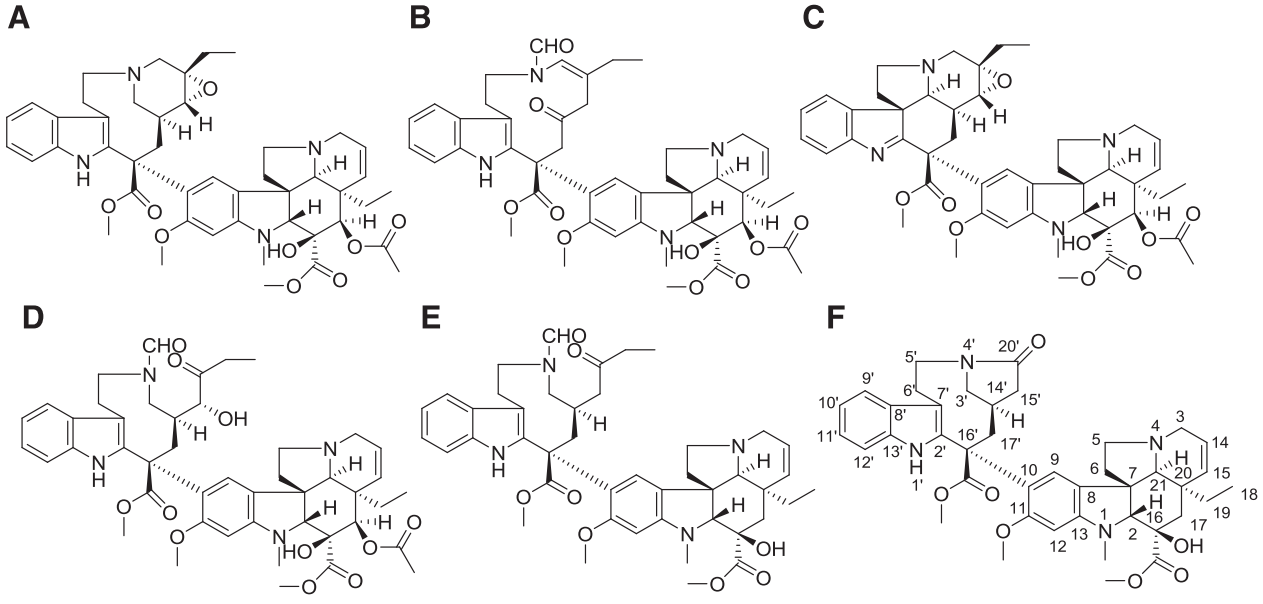

Fig. 1. Chemical structures of **A**, leurosine; **B**, catharine; **C**, cycloleurosine; **D**, 15′-*R*-hydroxyvinamidine; **E**, 17-deacetoxyvinamidine; and **F**, cathachunine.