Phytomedicine 23 (2016) 705–713

Contents lists available at ScienceDirect

Phytomedicine

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

Curcumin hampers the antitumor effect of vinblastine via the inhibition of microtubule dynamics and mitochondrial membrane potential in HeLa cervical cancer cells

Jae-Wook Lee[a], Sojin Park[a], Sun Yeou Kim[b], Sung Hee Um[c,**], Eun-Yi Moon[a,*]

[a] Department of Bioscience and Biotechnology, Sejong University, Seoul 05006, Republic of Korea
[b] College of Pharmacy, Gachon University, #191 Hambakmoero, Yeonsu-gu, Incheon 406-799, Republic of Kore
[c] Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Kyunggi-do 16419, Republic of Korea

ARTICLE INFO

Article history:
Received 5 January 2016
Revised 12 March 2016
Accepted 24 March 2016

Keywords:
Curcumin
Vinblastine
Microtubule
Mitochondrial membrane potential
ROS, Antitumor activity

ABSTRACT

Background: Curcumin, a major component of curry powder, which is a natural polyphenol product extracted from rhizoma curcumae longae, interacts with a specific binding site on microtubules. Vinblastine is an antitumor drug that induces microtubule depolymerization.
Purpose: We investigated whether curcumin influences the antitumor effect of vinblastine in HeLa human cervical cancer cells.
Study design: Changes in microtubule filaments were visualized by immuno-staining. Cell death was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) or water-soluble tetrazolium(WST) assay. Apoptotic cell formation was assessed by flow cytometry after staining cells with propidium iodide(PI) and/or Annexin V or with 6-diamidino-2-phenylindole(DAPI). Reactive oxygen species(ROS) were also measured by flow cytometry using dichloro-dihydro-fluorescein diacetate(DCF-DA). JC-1 was used to determine mitochondrial membrane potential (MMP).
Results: When cells were pretreated with curcumin, microtubule filaments were disordered. Vinblastine-induced microtubule depolymerization and cell death were reduced in HeLa human cervical cancer cells pretreated with curcumin compared to the control. The decrease in cell death was much greater in cells pretreated with curcumin compared to cotreatment or post-treatment. DNA condensation by vinblastine was also decreased in curcumin-pretreated cells. Curcumin reduced ROS production by vinblastine. However, no changes in vinblastine-mediated microtubule depolymerization were detected upon N-acetylcysteine(NAC) treatment. In contrast, vinblastine-induced MMP collapse was inhibited by pretreatment with curcumin or NAC. These findings suggest that vinblastine-induced tumor cell death might be inhibited by curcumin via ROS-independent microtubule dynamics and ROS-dependent MMP collapse. It also suggests that microtubule dynamics could be necessary for the optimal antitumor activity of vinblastine. Our results suggest that patients treated with vinblastine should not consume curcumin.

Introduction

Microtubules, which consist of heterodimers of α- and β-tubulin, form the filamentous cytoskeleton of eukaryotic cells. Microtubules play important roles in a variety of cellular functions, including chromatin separation in cell division, intracellular trafficking, cell movement, and the formation of flagella and cilia. Microtubules are highly dynamic structures that undergo continuous

Abbreviations: ROS, Reactive oxygen species; MMP, Mitochondrial membrane potential; NAC, N-acetyl cysteine; PI, Propidium iodide; DAPI, 6-diamidino-2-phenylindole; DCF-DA, 2′,7′-Dichlorodihydrofluorescein diacetate; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl-carbocyanine iodide (mitoprobe™ Jaggregate); MTT, [3(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide]; WST-8, (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt).

* Corresponding author. Department of Bioscience and Biotechnology, Sejong University, 98 Kunja-Dong Kwangjin-Gu, Seoul 143-747, Korea, Tel. +82 2 3408 3768; fax. +82 2 466 8768.
** Corresponding author. Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Gyeonggi-do 16419, Korea; and Department of Health Sciences and Technology, SAIHST, Samsung Medical Center, Sungkyunkwan University, Seoul 06351, Korea, Tel. +82-31-299-6123; fax. +82-31-299-6109.
E-mail addresses: shum@skku.edu (S.H. Um), eunyimoon@sejong.ac.kr (E.-Y. Moon).

http://dx.doi.org/10.1016/j.phymed.2016.03.011
0944-7113/

polymerization and depolymerization in the cytoplasm (Desai and Mitchison, 1997). Microtubules are also an attractive therapeutic target for the development of anticancer drugs (Bhalla, 2003), such as vinca alkaloids, taxanes, epothilones, and eribulin (Gigant et al., 2005; Jordan and Wilson, 2004).

Vinblastine is a tubulin-targeting agent that perturbs polymerization, which induces cell death (Gigant et al., 2005). Cancer cell apoptosis due to vinblastine is also mediated by the production of reactive oxygen species (ROS) (Chiu et al., 2012). The use of tubulin-targeting agents is limited by the development of resistance to anticancer drugs, which is induced by tubulin isotype mutation and the alteration of microtubule dynamics (Cheung et al., 2010; Natarajan and Senapati, 2012). However, little is known about the induction of drug resistance to vinblastine-mediated apoptosis in tumor cells.

Curcumin, a natural product targeting microtubules, is the most studied dietary phytochemical, which originates from the rhizome of *Curcuma longa* Linn (Zingiberaceae) (Gupta et al., 2006). It was recently revealed that curcumin interacts with a specific binding site on microtubules and suppresses their dynamic instability in many types of cancer cells (Banerjee et al., 2010; Chakraborti et al., 2011; Gupta et al., 2006). Curcumin has anticancer activity regarding tumor initiation, proliferation, progression, metastasis, invasion, and angiogenesis (Kawamori et al., 1999; Kunnumakkara et al., 2008). It is also an antioxidant, showing various chemotherapeutic effects, such as analgesic, anti-inflammatory, antiseptic, and antimicrobial activities (Ak and Gulcin, 2008; Anand et al., 2008; Sa and Das, 2008). However, the changes in the anticancer effects of vinblastine in tumor cells pretreated with curcumin as an antioxidant to scavenge ROS have not been clarified.

It was reported that the combined treatment of chemotherapeutics with dietary phytochemical agents could sensitize cancer cells to apoptosis and reduce side effects (Sak, 2012). For instance, curcumin has been shown to augment paclitaxel-induced apoptosis in MDA-MB-435 (Aggarwal et al., 2005), HeLa (Bava et al., 2005), LN18, and U138MG cells (Hossain et al., 2012), and to increase the anticancer effect of trichostatin A in MDAMB435eB and SK-Br3 cells (Yan et al., 2013). While curcumin is antagonistic to paclitaxel, it is additive to vinblastine in MCF-7 cells (Banerjee et al., 2010). This suggests that the anticancer effect of tubulin-targeting agents varies depending on the experimental conditions, such as tumor cell type, duration of incubation, and time of curcumin treatment. Although tubulin-targeting vinblastine is effective in various types of tumors, side effects are limitation to use it. In addition, cervical cancer is one of the most commonly diagnosed cancers in less developing countries and the fourth most common cancer in women worldwide (Ferlay et al., 2015). However, little is known about whether curcumin influences the anticancer effect of vinblastine through the alteration of microtubule dynamics or a decrease in ROS production in cervical cancer cells. Therefore, we investigated whether curcumin can contribute to the development of drug resistance to vinblastine by using HeLa human cervical cancer cells.

Materials and methods

Reagents

MTT [3(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide], Premix WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-*2H*-tetrazolium, monosodium salt), DCF-DA (2′,7′-Dichlorodihydrofluorescein diacetate), trypan blue solution, and curcumin were purchased from the Sigma-Aldrich (St. Louis, MO). Antibodies reactive with α-tubulin were obtained from Sigma-Aldrich (St. Louis, MO). MitoProbe™ J-aggregate (JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl-carbocyanine iodide) and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Life Technologies (Grand Island, NY). Except where indicated, all other materials are obtained from Sigma-Aldrich (St. Louis, MO).

Cell culture

HeLa human cervical cancer cells and B16F10 mouse melanoma cells were obtained from the Korea Research Institute of Bioscience and Biotechnology (KRIBB) cell bank (Daejeon, Korea). Cells were maintained and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA), 2 mM L-glutamine, 100 U/ml penicillin and 100 U/ml streptomycin. Then, cells were incubated at 37 °C in an atmosphere of humidified normoxia incubator with 5% CO_2 and 95% air (Lee et al., 2015; Ryu et al., 2015).

Animals

Adult male C57BL/6 mice (21–23 g, body weight) were housed under constant temperature (22 ± 2°C) and humidity with a 12 h light/dark cycle and with free access to chow and water. All mouse experiments were carried out in strict accordance with the guidelines of the Institutional Animal Care and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Maryland, USA). The protocol was approved by the Committee on the Ethics of Animal Experiments of Sejong University (Permit Number: SJ20120604-E3).

Cytotoxicity assay

Cell survival was quantified by using colorimetric assay described for measuring intracellular succinate dehydrogenase content with MTT (Yang et al., 2014) or Premix WST-8. Confluent cells were cultured with various concentrations of each reagent for 24 h. Cells were then incubated with 50 μg/ml of MTT at 37 °C for 2 h. Formazan formed by MTT were dissolved in dimethylsulfoxide (DMSO). Optical density (OD) was read at 540 nm. Otherwise, cells were also incubated with 10 μl of Premix WST-8 at 37 °C for 2 h. OD was read at 450 nm for water-soluble formazan formed by Premix WST-8.

Measurement of caspase 3 activity

Cells were harvested and incubated with lysis buffer [50 mM HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), pH 7.4, 5 mM CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), 5 mM DTT (dithiothreitol)] for 20 min on ice. Then, lysates were centrifuged by 16,000 xg for 10 min at 4°C. Supernatant of 5 μl was mixed with reaction buffer [20 mM HEPES, pH 7.4, 0.1% CHAPS, 5 mM DTT, 2 mM EDTA (ethylenediaminetetraacetic acid)] containing 200 μM of Ac-DEVD-pNA, caspase 3 substrate. After the incubation for 2 h, optical density(OD) was read at 450 nm. Caspase 3 activity was normalized with protein concentration.

In vivo *tumor growth*

Mice were acclimatized for 7 days before the xenograft with B16F10 mouse melanoma cells. To examine the effect of curcumin on antitumor activity of vinblastine, B16F10 cells were cultivated *in vitro* in log phase. 200,000 B16F10 cells were suspended in 50 μl sterile saline and subcutaneously injected into C57BL/6 mouse skin according to previous method modified (Lee et al., 2015). Beginning on the day of tumor volume at 60 mm^3, mice randomly divided into four groups (control group, 4 mg/kg