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Cucurbitacin-I induces hypertrophy in H9c2 cardiomyoblasts through activation of autophagy via MEK/ERK1/2 signaling pathway

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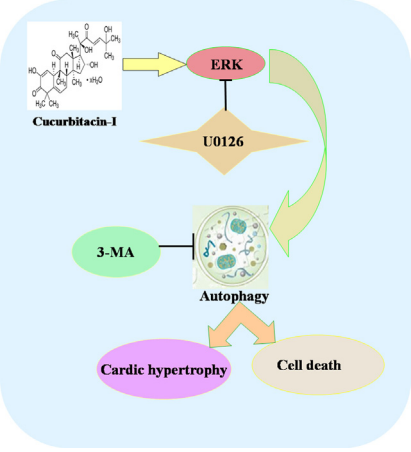
HIGHLIGHTS

- Cucurbitacin-I, a triterpenoids, has diverse pharmacological and biological activities.
- Cucurbitacin-I induced cardiotoxicity (hypertrophy and death) in H9c2 cells.
- Cucurbitacin-I induced strong autophagy, hypertrophy and apoptosis in H9c2 cells.
- Cucurbitacin-I induced cardiotoxicity via an ERK-autophagy dependent pathway.

GRAPHICAL ABSTRACT



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ABSTRACT

Cucurbitacin-I, a natural triterpenoids initially identified in medicinal plants, shows a potent anticancer effect on a variety of cancer cell types. Nevertheless, the cardiotoxicity of cucurbitacin-I has not heretofore been reported. In this study, the mechanisms of cucurbitacin-I-induced cardiotoxicity were examined by investigating the role of MAPK-autophagy-dependent pathways. After being treated with 0.1–0.3 μM cucurbitacin-I for 48 h, H9c2 cells showed a gradual decrease in the cell viabilities, a gradual increase in cell size, and mRNA expression of ANP and BNP (cardiac hypertrophic markers). Cucurbitacin-I concentration-dependent apoptosis of H9c2 cells was also observed. The increased apoptosis of H9c2 cells was paralleling with the gradually strong autophagy levels. Furthermore, an autophagy inhibitor, 3-MA, was used to block the cucurbitacin-I-stirred autophagy, and then the hypertrophy and apoptosis induced by 0.3 μM cucurbitacin-I were significantly attenuated. In addition, cucurbitacin-I exposure also activated the MAPK signaling pathways, including ERK1/2, JNK, and p38 kinases. Interestingly, only the ERK inhibitor U0126, but not the JNK inhibitor SP600125 and p38 MAPK inhibitor SB203580, weakened the induction of 0.3 μM cucurbitacin-I in hypertrophy, autophagy and apoptosis. Our findings suggest

Abbreviations: MAPK, mitogen-activated protein kinase; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; 3-MA, 3-methyladenine; DMSO, dimethyl sulfoxide; WGA, wheat germ agglutinin.

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that cucurbitacin-I can increase the autophagy levels of H9c2 cells, most likely, through the activation of an ERK-autophagy dependent pathway, which results in the hypertrophy and apoptosis of cardiomyocytes.

1. Introduction

Cancer remains an important public health concern in the world (Ramaswami et al., 2013). Although surgical resection and radiation remain the most common strategy for cancer therapy, anticancer agents are equally important for its convenient and efficient when used alone or in combination with surgery or radiotherapy (Huitink and Teoh, 2013; Saijo et al., 2003). Therefore, developing new effective anticancer drugs is still an important strategy in tumor therapy (Saijo et al., 2003). However, cardiotoxicity, a common complication of many anti-cancer agents, remains a major limitation and potential risk of cardiac dysfunction, strongly impacting the quality of life and the overall survival (Saijo et al., 2003; Double et al., 2002; Curigliano et al., 2012). Cardiotoxicity may occur during or shortly after treatment (within days or weeks), or it may become evident a long period after completion of anticancer therapy (Curigliano et al., 2012). A variety of antitumor drugs including anthracyclines, antimetabolites, cyclophosphamide and newer targeted agents have been eventually confirmed to cause cardiomyopathy (Curigliano et al., 2012; Stortecky and Suter, 2010). Strategies to prevent or mitigate cardiac damage from anticancer drugs are needed to provide the best cancer care (Saijo et al., 2003; Double et al., 2002; Stortecky and Suter, 2010).

Cucurbitacin-I, a natural triterpenoid (Fig. 1) purified from the fruit extract of cucurbitaceae family plants, such as cucumber, has been used in traditional medicine for its antipyretic, analgesic, anti-inflammatory and antimicrobial actions (Lee et al., 2010). Recently, accompanied by discoveries showing that cucurbitacins are the strong signal transducers and activators of transcription-3 (STAT3) inhibitors, the antitumor effect of cucurbitacins has received more and more attention (Lee et al., 2010; Alghasham, 2013). As one of the 12 categories of cucurbitacins, cucurbitacin-I has a potent anticancer effect on several types of cancer cells, including breast cancer, lung cancer, and glioma, which is comparable to cucurbitacin-B and −D, both of them suppress the proliferation of a variety of cancer cells in vitro and in vivo (Lee et al., 2010; Alghasham, 2013; Chen et al., 2012). Cucurbitacins-induced toxicities in patients present with symptoms including diarrhea, ematemesis, hypotension and gastrointestinal mucosal injury (Ho et al., 2014), while it is still not clear whether cucurbitacins induce cardiac toxicity, which is a common side effect of other anticancer agents (Alghasham, 2013; Chen et al., 2012; Ho et al., 2014).

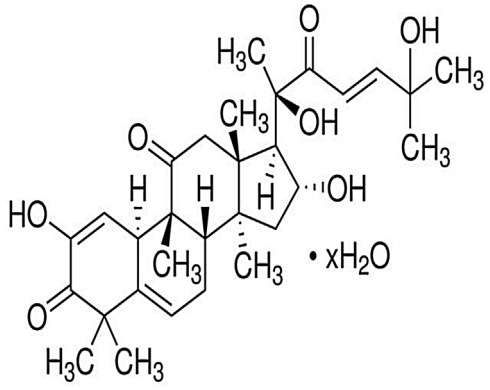

Fig. 1. Chemical structure of Cucurbitancin-I.
Cucurbitacin-I is a natural cell-permeable triterpenoid purified from cucurbitaceae plants.

Autophagy, an evolutionarily conserved catabolic degradative process, plays a vital role in cardiac physiology and is critical to the survival of both tumor cells and heart cells (White et al., 2015; Li and Hill, 2014). Autophagy has been proved to be a mechanism that various cancer cells withstand metabolic stress and cancer therapy cytotoxicity, and it is a promising target for cancer therapy (White et al., 2015). Many of the chemotherapeutics currently in clinical use alter autophagy levels in cancer cells or cardiomyocytes, and autophagy plays an important role in cardiotoxicity caused by these drugs (Li and Hill, 2014). Elucidation of the biology of autophagy will help prevent or treat chemotherapy-induced cardiotoxicity (White et al., 2015; Li and Hill, 2014). Additionally, development of future autophagy-targeting anti-cancer drugs warrants caution for cardiac side effects, especially when used in combination with other drugs known to affect autophagy levels (Curigliano et al., 2016; White et al., 2015; Li and Hill, 2014).

Yuan G (Yuan et al., 2014), Zhang T (Zhang et al., 2012) and their colleagues have observed that cucurbitacin-I induced robust autophagy in a variety of cancer cells. Little is known presently about whether cucurbitacin-I exposure induces the cardiomyocyte autophagy in the setting of chemotherapy, and whether autophagy plays an important role in the cucurbitacin-I-induced cardiac toxicity (Alghasham, 2013; Chen et al., 2012). In the current study, the myoblast cell line H9c2 cells, derived from embryonic rat heart, were challenged by cucurbitacin-I to study its potential mechanism of inducing autophagy-dependent cardiotoxicity.

2. Materials and methods

2.1. Materials

Cucurbitacin-I, Dimethyl sulfoxide (DMSO) and 3-methyladenine (3-MA) were purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA); WGA, Alexa FluorR 488 conjugate, TRIzol Reagent, lipofectamin TM2000, and the fluorescein isothiocyanate (FITC) Annexin V and propidium iodide (PI) kit for apoptosis detection from Invitrogen (Carlsbad, CA, USA); iScript™ cDNA Synthesis Kit and iQTM SYBR® Green Supermix from Bio-Rad Laboratories, Inc. (Hercules, CA, USA); Hoechst 33258 from KeyGen Biotech. CO. Ltd (Shang Hai, China); anti-Extracellular signal regulated protein kinase (ERK), anti-phosphor-ERK (Thr202/Tyr204), anti-c-Jun NH2-terminal kinase (JNK), anti-phosphor-JNK, anti-p38, anti-phosphor-p38, anti-caspase3, anti-LC3 B and anti-β-actin antibodies from Cell Signaling Technologies (Beverly, MA, USA); 4′6′-diamidino-2-phenylindole (DAPI) from Roche (Roche Applied Science, Indianapolis, IN, USA), U0126-EtOH (a ERK inhibitor) from Selleckchem (Houston, TX, USA), SP600125 (a JNK inhibitor), and SB203580 (a p38 Mitogen-Activated Protein Kinase (MAPK) inhibitor) were from Calbiochem (Cambridge, MA, USA). All pairs of PCR primers were synthesized by Shenggong Biotechnology (Shanghai, China). Other chemicals and reagents were of analytical grade.