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Enhanced autophagic activity of artocarpin in human hepatocellular carcinoma cells through improving its solubility by a nanoparticle system

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the most common liver cancer worldwide, with poor prognosis and resistance to chemotherapy. This gives novel cancer treatment methods an overwhelming significance. Natural products offer great resources of developing new and effective chemopreventive or chemotherapeutic agents. *Artocarpus communis* extracts and its active constituent, prenylated flavonoid artocarpin induce human hepatocellular carcinoma cell death. However, the poor water solubility drawbacks of artocarpin restrict its clinical application and bioavailability.
Purpose: This study developed the artocarpin nanoparticle system to overcome the poor water solubility drawbacks and investigated the improvement of therapeutic efficacy of artocarpin by adopting novel nanoparticle delivery strategy.
Methods: Antiproliferative activity of artocarpin was evaluated by MTT assay. Cell morphology observation by microscope, DNA fragmentation assay, cell cycle analysis, Annexin V apoptosis cell staining, monodansylcadaverine and acridine orange staining and immunoblot analysis were used to evaluate the induction of autophagy by artocarpin. The determination of particle size, amorphous transformation, hydrogen-bond formation, yield, encapsulation efficiency and the solubility study were used to investigate the solubility enhancement mechanism of artocarpin.
Results: The present study demonstrates that the anticancer effect of artocarpin in HepG2 and PLC/PRF/5 hepatoma cells is mediated through the autophagic cell death mechanism. Results also demonstrated that artocarpin nanoparticles enhanced the solubility of artocarpin by reducing particle size, transforming high energy amorphous state, and forming hydrogen bond with excipients. Additionally, ArtN exhibited better autophagic cytotoxicity compared to free artocarpin.
Conclusion: This work reveals the antihepatoma activity of artocarpin by inducing autophagic cell death and the improvement of therapeutic efficacy of artocarpin by adopting novel nanoparticle delivery strategy. The research provided a basis of ArtN could be explored as a low-dose alternative of artocarpin in anticancer treatment and research applications.

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Introduction

Prenylated flavonoid artocarpin (Fig. 1) can be isolated from *Artocarpus communis* and could be considered as an indicator of the anticancer potential of *A. communis* extracts (Tzeng et al. 2014). *A. communis* is primarily grown in Southeast Asia and offers economic value as a multipurpose crop that is widely used in food, traditional medicine, and the timber industry. Artocarpin is known to possess multiple pharmacological activities, such as anticancer, 5-alpha-reductase inhibition (Jagtap and Bapat 2010), and skin-protective effect from UVB-induced damage (Lee et al. 2013). Previous research showed that artocarpin possess cytotoxic effect toward several cancer cells, however, no antihepatoma effect was reported until our discovery of *A comuunis* extracts possess antihepatoma effect in HepG2 and PLC/PRF/5 cells (Tzeng et al. 2014).

Flavonoid compounds have been shown to possess a wide range of biological and pharmacological activities in various studies. However, the low water solubility of flavonoid limits their clinical use. Mallick et al. had demonstrated that the low water solubility and poor dissolution property of compounds would influence their bioavailability (Mallick et al. 2007). The solubility of artocarpin is 2.6 mg/l in water, but it is soluble in organic solvents such as DMSO or ethanol. DMSO and ethanol are commonly used in researches to dissolve hydrophobic compounds. However, the safety of high concentration of DMSO or ethanol is questionable due to their risk of toxicity (Lee et al. 2014). The modification of drug delivery system, such as nanoparticle, is applicable to overcome the low solubility drawbacks of hydrophobic compounds and reduce the required dosage to enhance drug efficacy (Lee et al. 2012). Nanoparticle is a stable colloidal particle system with the size in a range of 100 nm and it is wildly used in drug delivery system for water-insoluble compounds. It can be composed of numerous materials and can be produced by several methods such as nanoprecipitation, wet milling, or high pressure homogenization (Guo and Huang 2014).

Autophagy is a major intracellular degradation mechanism that functions to maintain the basic energy level of cells, and is activated under cell stress conditions to promote survival, or, lead to type II programmed cell death if overexpressed. Autophagy acts as a double-edged sword as it may induce tumor suppression or tumor survival under the treatment of chemotherapeutic agents (White and DiPaola 2009). However, autophagy induced by chemotherapeutic drugs is more likely to result in tumor inhibition than tumor growth (Janku et al. 2011). Autophagy shows distinct features from apoptosis by lacking the classical characteristics of apoptosis and characterized by the presence of double-membrane autophagosomes and the activation of autophagy-related genes that control the formation of autophagic vesicles (Parzych and Klionsky 2014). Studies of natural products showed that the induction of autophagy is an important mechanism to exert their cytotoxic effects (Sun et al. 2013).

The antihepatoma mechanism of artocarpin nanoparticle system and the solubility enhancement of artocarpin by nanoprecipitation have not yet been elucidated. The present study demonstrates that the anticancer effect of artocarpin in HepG2 and PLC/PRF/5 hepatoma cells is mediated through the autophagic cell death mechanism. We also establish a novel artocarpin nanoparticle system to overcome its poor water solubility and ameliorate its anticancer effect compared to free artocarpin.

Materials and methods

Chemicals

Artocarpin was isolated from the dichloromethane fraction of *A. communis* as mentioned previously (Tzeng et al. 2014). 1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide (MTT), polyvinylpyrrolidone (PVP), monodansylcadaverine (MDC), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), penicillin G, streptomycin, and amphotericin B were purchased from GIBCO BRL (Gaithersburg, MD). RNase A was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA), and acridine orange (AO) was purchased from Merck Millipore (Darmstadt, Germany). Polyclonal antibodies against Bcl-2, p53, p-p53, caspase-3, caspase-8, caspase-9, β-actin, and horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (CA, USA). PARP (poly ADP-ribose polymerase) cleavage site antibody was purchased from Merck Millipore (Merck KGaA, Darmstadt, Germany). LC3 antibody was purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). All other chemical reagents were of analytical grade.

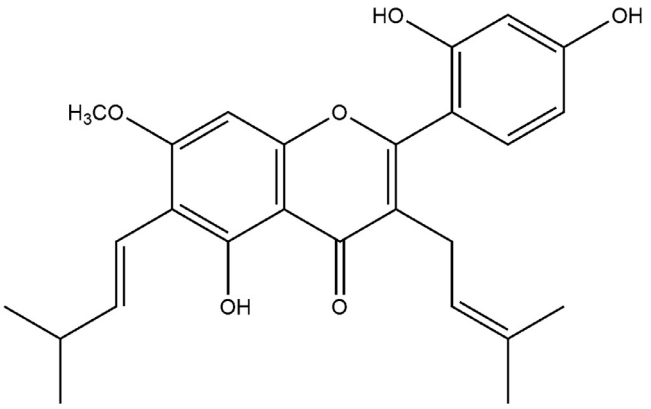

Fig. 1. Structure of artocarpin.

Plant material and extraction

The heartwood of *A. communis* was purchased from Tainan District Agricultural Research and Extension Station. The plant species was authenticated by Dr. Ming-Hong Yen of the Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan. The voucher specimen of *A. communis* J.R. Forst. & G. Forst was deposited in the Herbarium of the Department of Fragrance and Cosmetic Science, Kaohsiung Medical University, Kaohsiung, Taiwan (Voucher specimen number: 2001-ACHW).

Cell line and cell culture

Human hepatoma cell lines HepG2 and PLC/PRF/5 were purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan). Cells were maintained in high-glucose DMEM addition with 10% fetal bovine serum and incubated under standard cell culture conditions at 37 °C and 5% CO_2 in a humidified incubator.

Cell viability and cell morphology observation

The effect of artocarpin on cell viability was evaluated by MTT assay as previously described (Tzeng et al. 2014). The morphology of carcinoma cells was captured by a microscope (Nikon, Japan) as previously described (Tzeng et al. 2015).

DNA fragmentation assay

Cells were treated with artocarpin for 24 h and then collected by trypsinization. Positive control was prepared by exposing cells to UV light for 10 min and incubating for another 6 h. The ApopLadder ExTM Kit (Takara Bio Inc., Otsu, Japan) was used to