Phytomedicine 20 (2013) 904–912

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Antitumor activity of caffeic acid 3,4-dihydroxyphenethyl ester and its pharmacokinetic and metabolic properties

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ARTICLE INFO

Keywords:
Hydroxyphenethylester of caffeic acid
Antitumor
Pharmacokinetics
Metabolites
HPLC
LC/MS

ABSTRACT

Caffeic acid 3,4-dihydroxyphenethyl ester (CADPE), a natural polyphenol from *Sarcandra glabra*, has potent *in vitro* anticancer activity through multiple targets. This study investigated its *in vivo* anticancer efficacy and its pharmacokinetic and metabolic characteristics. CADPE at any of the dosage regimes (ip 2.5 mg/kg at an interval of 7 h, 12 h, or 24 h for eight days) significantly decreased tumor growth in hepatoma H22 and sarcoma S180 tumor-bearing mice. CADPE also significantly inhibited H22-induced acute ascites development. The *in vivo* anticancer efficacies of CADPE in these tumor models were equivalent to those of 5-fluorouracil (10 mg/kg, ip) and cyclophosphamide (10 mg/kg, ip), and CADPE did not show any toxicity. A high performance liquid chromatography method with the aid of liquid chromatography/mass spectrometry was established and validated for the pharmacokinetic and metabolic studies of CADPE. CADPE was detected in blood and the organs including liver, kidney, heart, spleen, and brain 1 min after tail intravenous administration, indicating that CADPE was able to quickly distribute to these organs. CADPE was quickly hydrolyzed both in mice and *in vitro* mice plasma, but was much stable *in vitro* human plasma, suggesting a better bioavailability of CADPE in human than in mice. The major metabolites of CADPE in mice were caffeic acid, hydroxytyrosol, and a CADPE glucuronide. This was the first time to reveal the pharmacokinetic and metabolic characteristics of CADPE. Taken together, CADPE had potent *in vivo* antitumor activity and was able to rapidly reach the body organs and to be hydrolyzed in blood to anticancer agents of caffeic acid and hydroxytyrosol. This study suggested that CADPE has the potential for the treatment of cancers and is worthy of further study.

Introduction

Sarcandra glabra (Thunb) Nakai (Chloranthaceae family) is a renowned traditional Chinese medicine for its antibacterial, anti-inflammatory, and antitumor effects. The whole plant and the preparations made from the whole plant of *S. glabra*, such as Zhongjiefeng extract, Zhongjiefeng tablets, and Xuekang capsules, are mainly used for the treatment of pharyngolarygitis, acute influenza, pneumonia, shigellosis, cellulitis, appendicitis, thrombocytopenia, leukoderma vitiligo, and abscess (National Commission of Chinese Pharmacopoeia 2010). In recent decades, Zhongjiefeng injections made from *S. glabra* extract have been used to treat cancers including gastric cancer, colon cancer, pancreatic cancer, and leukemia. Although the chemical constituents of *S. glabra* have been extensively investigated (Fu et al. 2011; Xu et al. 2011), its anticancer ingredients are still unknown. Our group first isolated an anticancer polyphenolic ester of caffeic acid 3,4-dihydroxyphenethyl ester (CADPE, **1**, Fig. 1) from *S. glabra* and revealed its broad-spectrum *in vitro* antitumor activity (Lian and Zhang 2006, 2009a,b; Zhang et al. 2010). The tested 59 human cancer cell lines from leukemia and nine different solid tumors including colon cancer, gastric cancer, breast cancer, CNS cancer, ovarian cancer, melanoma, lung cancer, renal cancer, and prostate cancer are sensitive to CADPE. Because of a very low concentration of CADPE in the plants, we have recently developed an efficient method to generate CADPE in high yields to facilitate an ongoing preclinical investigation on its potential for the treatment of cancer (Zhang et al. 2010; Zhang 2011).

Recent studies have demonstrated that CADPE produced anticancer activity through modulating multiple targets. (1) CADPE suppressed tumor growth and angiogenesis by inhibiting the

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http://dx.doi.org/10.1016/j.phymed.2013.04.002

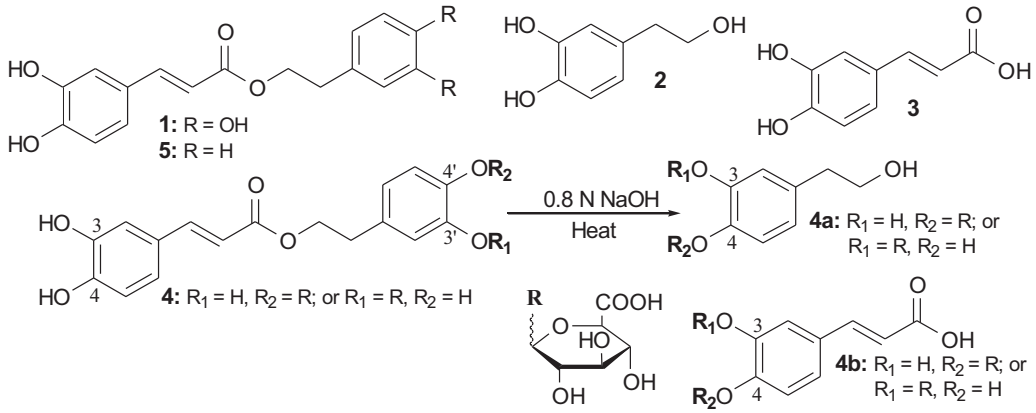

Fig. 1. Structures of compounds **1**–**5** (**1**: CADPE; **2**: hydroxytyrosol; **3**: caffeic acid; **4**: CADPE-4′-*O*-*β*-D-glucuronopyranosyl acid, or CADPE-3′-*O*-*β*-D-glucuronopyranosyl acid; **4a**: hydroxytyrosol-4-*O*-*β*-D-glucuronopyranosyl acid, or hydroxytyrosol-3-*O*-*β*-D-glucuronopyranosyl acid; **4b**: caffeic acid-4-*O*-*β*-D-glucuronopyranosyl acid, or caffeic acid-3-*O*-*β*-D-glucuronopyranosyl acid; **5**: CAPE).

activity of signal transducers and activated transcription 3 (STAT3), the expression of hypoxia inducible factor-1 alpha (HIF-1 alpha), and the vascular endothelial growth factor in a mouse xenograft model implanted with Caki-I human renal carcinoma cells (Jung et al. 2007); (2) CADPE inhibited cyclin D1 expression in hepatocellular carcinoma cells by blocking both IL-6-mediated STAT3 activation and recruitment of STAT3 to the cyclin D1 promoter (Won et al. 2010); (3) CADPE significantly inhibited PMA-stimulated gastric carcinoma cell invasion and matrix metalloproteinase-9 expression by FAK/MEK/ERK-mediated AP-1 activation (Han et al. 2010); and (4) CADPE induced cancer cell senescence by regulating the Twist1-modulated p53-$p21^{WAF1/CIP1}$ and $p16^{INK4a}$ signaling pathways (Dong et al. 2011). Those targets and pathways that CADPE targeted have been shown to play crucial roles in tumorigenesis, tumor development and progression. It has been proposed that modulating multiple cellular targets and signal pathways could be beneficial for the prevention and treatment of complex human diseases such as cancer (Frantz 2005; Keith et al. 2005; Morphy and Rankovic 2007; Goel et al. 2008). The anticancer mechanism profiles of CADPE suggest its potential for the prevention and treatment of cancers.

Whether CADPE could be developed into an anticancer drug is dependent on not only its *in vivo* anticancer activity and but also its bioavailability. However, only one study reported that CADPE inhibited tumor growth in Caki-I human renal tumor xenograft animal model (Jung et al. 2007), and the pharmacokinetic and metabolic characteristics of CADPE are completely unknown so far. Therefore, the current study focused on the anticancer activity of CADPE using *in vivo* animal models and the pharmacokinetic and metabolic properties of CADPE in mice and *in vitro* human and mice plasma by a validated high performance liquid chromatography (HPLC) method with the aid of liquid chromatography/mass spectrometry (LC/MS). The results from this study indicated that CADPE had potent *in vivo* anticancer efficacy and was able to rapidly reach the body organs and to be hydrolyzed in blood to anticancer agents of caffeic acid and hydroxytyrosol. This study together with previous investigations demonstrated that CADPE is a promising anticancer natural compound and worthy of further translational study.

Materials and methods

Animals and tumor cells

Male ICR mice (24–26 g) were purchased from the Zhejiang Experimental Animal Center (Certificate No. SCXK-ZHE 2008-0033, Hangzhou, China). All animals were housed in a standard environment under controlled conditions (24–26 °C, a 12 h light/dark cycle) with free access to food and water. The animals were allowed to acclimate for at least one week before their use. All procedures involving animals and their care were approved by the Zhejiang University Animal Experimentation Committee and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All surgery was performed under urethane anesthesia, and all efforts were made to minimize suffering.

Mouse hepatoma H22 and mouse sarcoma S180 cell lines were purchased from the Chinese Academy of Sciences (Shanghai, China) and preserved in the Division of Pharmacodynamics and Drug Development, Zhejiang University (Hangzhou, China).

H22 or S180 was maintained in the ascitic form through sequential passages in male ICR mice, by means of weekly intraperitoneal (ip) transplantations of 1×10^7 tumor cells in 0.2 ml. To establish the tumor-bearing mouse model, H22 or S180 cells with ascites were harvested and injected subcutaneously into the right armpit region of the mice.

Chemicals and reagents

Caffeic acid 3,4-dihydroxyphenethyl ester (CADPE, **1**, 98.6%) and hydroxytyrosol (**2**, 98.8%) were synthesized by using reported method (Zhang 2011). The structures (Fig. 1) of **1** and **2** were identified by nuclear magnetic resource (NMR) and high resolution mass spectrometry (HRMS) analysis and their purity was determined by HPLC. CADPE was dissolved in 10% hydroxypropyl-*β*-cyclodextrin to make a final concentration of 4.8 or 6.7 mg/ml for *in vivo* and *in vitro* assay. Caffeic acid (**3**, ≥98.0%), *β*-glucuronidase (Type HP-2, 7500 U/ml), glucuronic acid, urethane, and HPLC grade acetonitrile were purchased from Sigma. Cyclophosphamide (CTX) was purchased from HengRui Medicine Co Ltd (Jiangsu, China). 5-Fluorouracil (5-FU) was purchased from XudongHaipu Co Ltd (Shanghai, China). Sodium heparin (150 IU/mg, Biosharp) was obtained from AwardBio Co Ltd (Shanghai, China). De-ionized water was purified by a Milli-Q system (Millipore, Bedford, MA, USA). Other analytical grade solvents of methanol (MeOH), acetic acid, formic acid, and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co Ltd (Shanghai, China). Octadecyl silica (ODS, Cosmosil $75C_{18}$-Prep) and silica gel TLC plates were purchased from Microwants (Suzhou, China).

Animal models and drug treatments

One week after the mice acclimated the environment, about 1×10^7 H22 and S180 tumor cells were injected intraperitoneally