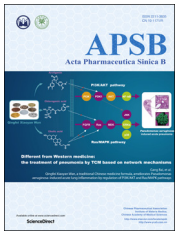
Acta Pharmaceutica Sinica B ▮▮▮▮;▮(▮):▮▮▮–▮▮▮

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Synthesis of hydroxycinnamic acid derivatives as mitochondria-targeted antioxidants and cytotoxic agents

Jiyu Li[a,1], Dian He[a,1], Baitao Wang[a], Ling Zhang[a], Kun Li[a], Qinjian Xie[b], Lifang Zheng[a,*]

[a]*School of Pharmacy, Lanzhou University, Lanzhou 730000, China*
[b]*Gansu Corps Hospital of Chinese People Armed Police Forces, Lanzhou 730000, China*

Received 7 March 2016; accepted 13 May 2016

KEY WORDS

Mitochondrial dysfunction;
Hepatocellular carcinomas;
Hydroxycinnamic acids;
Antiproliferative activities;
Mitochondrial permeability transition pore

Abstract In order to develop agents with superior chemopreventive and chemotherapeutic properties against hepatocellular carcinomas, mitochondria-targeted hydroxycinnamic acids (MitoHCAs) were synthesized by conjugation with a triphenylphosphonium cation. These synthetic compounds were evaluated for their antioxidant activities in hepatic mitochondria, including against OH•- and ROO•-induced lipid peroxidation. H_2O_2 production was decreased significantly by increasing glutathione peroxidase and catalase activities. In addition, cell proliferation data from three cell lines (HepG2, L02 and WI38) indicated that the MitoHCAs were selective for cancer cells. Interestingly, the MitoHCAs both with or without Ca^{2+} triggered mitochondrial dysfunction by inducing mitochondrial swelling, collapsing the mitochondrial membrane potential and causing cytochrome *c* release. In particular, an inhibitor of the mitochondrial permeability transition pore (mPTP), cyclosporin A, attenuated mitochondrial damage and cell apoptosis, indicating that mPTP may be involved in the antiproliferative activity of MitoHCAs. Further studies focused on structural optimization of these compounds are onging.

*Corresponding author. Tel./fax: +86 931 8915686.
E-mail address: zhenglf@lzu.edu.cn (Lifang Zheng).
[1]These authors made equal contributions to this work.
Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.05.002

Please cite this article as: Li Jiyu, et al. Synthesis of hydroxycinnamic acid derivatives as mitochondria-targeted antioxidants and cytotoxic agents. *Acta Pharmaceutica Sinica B* (2016), http://dx.doi.org/10.1016/j.apsb.2016.05.002

1. Introduction

The liver is responsible for detoxifying noxious metabolites and requires substantial amounts of adenosine triphosphate (ATP) from mitochondria to fulfill this biological process. Prolonged exposure of the liver to detrimental xenobiotics causes oxidative damage to mitochondria[1,2]. The resulting defective mitochondria and decreased ATP synthesis may lead to the induction of hepatocellular carcinomas[3].

Hepatocellular carcinomas continue to be a major health concern and are ranked as the fifth most common cancer worldwide. In China, high incidence rates have accounted for 55% of the world's cases[4,5]. Typical treatments for hepatocellular carcinomas have included surgery, radiotherapy and chemotherapy, but current cure rates are not satisfactory[6]. Currently, mitochondria have been recognized as potential targets in cancer cells, as these organelles are a reservoir for proteins that promote apoptotic death when transported into the cytosol[7]. These findings underscore the importance of discovering mitochondria-targeted drugs that would protect healthy mitochondria from oxidative damage or selectively induce apoptosis in hepatocellular carcinomas, while simultaneously affecting as few healthy cells as possible.

Hence, mitochondria-targeted drugs have been developed using drug delivery carriers, such as the triphenylphosphonium (TPP) cation, mitochondria-targeted peptides, nanoparticles, cell penetrating peptides, etc[8]. The TPP cation allows for mitochondrial delivery via the electrical gradient across the mitochondrial inner membrane and has been developed by Murphy et al.[9]. Further, as a versatile therapeutic carrier, TPP-targeted drugs may exhibit selectivity towards tumor cells because cancer cells maintain a higher mitochondrial potential than non-malignant cells[10]. Additionally, because cancer mitochondria are structurally and functionally different from their normal counterparts, cancer cells display extensive metabolic reprogramming and are more susceptible to mitochondrial perturbations[7]. Therefore, mitochondria-targeted drugs have been synthesized based on active molecules, such as 1,4-naphthoquinone[11], resveratrol[12], coenzyme Q[13], etc.

In recent years natural products have generated significant interest as chemopreventive and chemotherapeutic agents[14,15]. Hydroxycinnamic acids (HCAs) and their derivatives are commonly isolated from Chinese medicinal herbs[16–18] and are found in some plant-derived food products[19]. Epidemiological studies have reported that coffee drinkers demonstrate a significant reduction in the risk for hepatocellular carcinomas when compared to non-drinkers[20]. Recently, caffeic acid was reported to protect rat liver from reperfusion injury *via* regulation of the *Sirt3*-mitochondrial respiratory chain pathway[21] and against *tert*-butyl hydroperoxide-induced oxidative hepatic damage[22]. Additionally, high doses of HCAs and their derivatives induced growth arrest and apoptosis in a variety of tumor cells by triggering either mitochondrial dysfunction or the mitochondria-dependent apoptotic pathway[23–25]. Despite the undoubted protective effects or anticancer efficacy, clinical trials have failed to establish a link between treatment with HCAs and cancer reduction, although this may be due to insufficient dosages to specific tissues[26,27].

In this study, mitochondria-targeted HCA derivatives (MitoHCAs) were synthesized (Scheme 1), where *p*-coumaric (*p*-CoA), caffeic (CaA), ferulic (FA) and cinnamic acid (CA) were conjugated to the TPP cation, consequently resulting in Mito*p*-CoA, MitoCaA, MitoFA and MitoCA, respectively. The synthesized MitoHCAs protected mitochondria from lipid peroxidation and decreased mitochondrial H_2O_2 levels by regulating antioxidant enzymes. In addition, they displayed high toxicity against human hepatoma HepG2 cells, but low toxicity toward two healthy cell lines—human hepatic L02 and diploid human fibroblasts WI38 cells. Further, MitoHCAs, both with and without Ca^{2+}, caused mitochondrial permeability transition pore (mPTP) opening, thereby collapsing the mitochondrial membrane potential (MMP) and causing cytochrome *c* (cyt *c*) release in isolated hepatic mitochondria, all of which initiate the apoptotic pathway[28]. Finally, cyclosporin A (CsA) attenuated MitoCaA-induced apoptosis and mitochondria damage.

2. Results and discussion

2.1. Synthesis

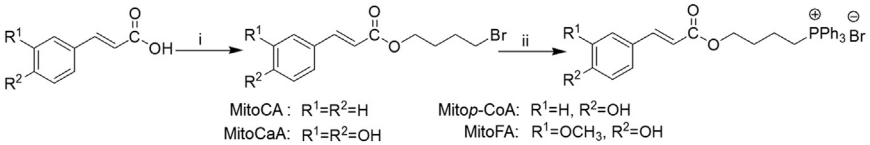
Synthesis of the MitoHCAs was performed as previously described (Scheme 1)[12,29]. The MitoHCAs were obtained in good yields (>70%) and were characterized by ^1H and ^{13}C NMR spectroscopy and HR-MS mass spectrometry (Figs. S1–S4). The purity was >95%, as determined by HPLC analysis (Fig. S5).

2.2. Inhibition of mitochondrial lipid peroxidation

As HCAs are excellent antioxidants, the synthesized MitoHCAs could act as novel inhibitors of mitochondrial lipid peroxidation. MitoCaA exhibited a dose-dependent suppression of lipid peroxidation causing full inhibition at ~6 μmol/L, while the parent compound CaA had no effect on lipid peroxidation induced by $FeCl_2$/ascorbate, which generates $OH^{\bullet}$ radicals *in situ*[30] (Fig. 1A). The inhibition potency of the synthesized MitoHCAs was measured: Mito*p*-CoA < MitoFA < MitoCaA (Fig. 1B). MitoCA and the control compounds (phosphonium salts butylTPP and CaA) were all ineffective at 6 μmol/L. Additionally, similar results were observed when mitochondria were treated with ROO• radicals and MitoHCAs (Fig. S3). Overall, these data suggest that the inhibitory potency of HCAs against mitochondrial lipid peroxidation was improved by addition of the TPP carrier.

2.3. Reduction of mitochondrial hydrogen peroxide levels and possible mechanisms

Supplementation with MitoHCAs protected liver mitochondrial lipids from exogenous ROS attack. In order to characterize this protection



Scheme 1 Synthesis of MitoHCAs. Reagents and conditions: (i) $Br(CH_2)_4Br$, Et_3N, Me_2CO, 50 °C, 4 h (55–65%); (ii) PPh_3, toluene, reflux, 36 h (70–85%).

Please cite this article as: Li Jiyu, et al. Synthesis of hydroxycinnamic acid derivatives as mitochondria-targeted antioxidants and cytotoxic agents. *Acta Pharmaceutica Sinica B* (2016), http://dx.doi.org/10.1016/j.apsb.2016.05.002