Journal of Ethnopharmacology 198 (2017) 331–337

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Antioxidative and anticancer properties of Licochalcone A from licorice

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ARTICLE INFO

Keywords:
LCA
Antioxidant
Anticancer
CAA

ABSTRACT

Ethnopharmacological relevance: Licochalcone A (LCA) is a characteristic chalcone that is found in licorice, which is a traditional medicinal plant. In traditional medicine, LCA possesses many potential biological activities, including anti-parasitic, anti-inflammatory and antitumor activities.

Aim of the study: To determine the antioxidant activity of LCA and, on this basis, to investigate the role of its anticancer activity.

Materials and methods: To validate the antioxidant activity of LCA, the proteins SOD, CAT and GPx1 were analyzed using western blotting and cellular antioxidant activity (CAA) assays. Oxidative free radicals are associated with cancer cells. Therefore, the anticancer activity of LCA was also evaluated. To assess the anticancer activity, cell viability assays were performed and apoptosis was evaluated. In addition, MAPK-related proteins were analyzed using western blotting.

Results: The experimental data showed that the EC_{50} of LCA is 58.79 ± 0.05 μg/mL and 46.29 ± 0.05 μg/mL under the two conditions tested, with or without PBS. In addition, LCA at a concentration of approximately 2–8 μg/mL can induce the expression of SOD, CAT and GPx1 proteins. Further, LCA inhibits the growth of HepG2 cells through cell proliferation arrest and the subsequent induction of apoptosis, and LCA attenuated the p38/JNK/ERK signaling pathway in a dose-dependent manner.

Conclusion: The results showed that LCA suppresses the oxidation of cells and markedly inhibits the proliferation of cancer cells. These findings confirm the traditional use of LCA in folk medicine.

1. Introduction

Medicinal plants and their extracts have been used extensively for centuries to treat many diseases. Among these natural compounds, antioxidants or free radical scavengers have received special consideration because of their pharmacological potential. Currently, although chemical synthetic antioxidants exhibit good resistance to oxidative damage, there is a growing consensus that the continuous use of chemical synthetic antioxidants in the body may be a health threat (Ho et al., 2000), and their use is restricted due to their carcinogenicity. Consequently, we should prioritize finding natural, effective and safe antioxidants that can protect the human body and slow the progress of many diseases, including cancer, which is associated with an increase in free radicals due to cell oxidation (Nandita and Rajini, 2004).

Licochalcone A (LCA) is a phenolic chalcone compound and a characteristic chalcone of licorice, which is the root of Glycyrrhiza inflata Batalin (Kwon et al., 2008), also named Gan-Cao in China. Licorice is widely accepted as an herbal medicine and as a traditional food in Asia. In China, licorice is listed in the Chinese Pharmacopoeia and the List of Herbal Materials and can thus be considered a health food under the Law on Food Hygiene by the State Food and Drug Administration. Currently, licorice remains one of the most commonly prescribed herbs for the treatment of various diseases, such as microbial infection, inflammation and cancer, and it is used for preparing a few famous Traditional Chinese Medicine (TCM) products, such as Naolejing Oral Liquid, Mistura Glycyrrhizae Composita and HuoxiangZhengqi Tincture. A safe daily dose of licorice can be as high as 60 g/day (Liu et al., 2014). The concentration of LCA in dried licorice (0.4–1% w/w) is approximately 4–10 mg/g, which renders into a concentration of approximately 240–600 mg of LCA as the daily dose (Zhang and Ye, 2009; Shibata, 2000). In addition to its use in food products, licorice has been used in pharmaceuticals and is prescribed in approximately 70% of traditional Japanese medicines (Kampo medicines). According to previous studies, LCA can trigger apoptosis or inhibit proliferation (Zhao et al., 2013; Kim et al., 2014; Hao et al., 2013; Shen et al., 2014) in various types of cancer cells (Yao et al.,

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http://dx.doi.org/10.1016/j.jep.2017.01.028
Received 1 November 2016; Received in revised form 3 January 2017; Accepted 16 January 2017
Available online 19 January 2017
0378-8741/

2014; Hao et al., 2015; Yang et al., 2014; Cho et al., 2014; Zeng et al., 2014; Park et al., 2015; Jiang et al., 2014; Yuan et al., 2013; Kim et al., 2015). To the best of our knowledge, when LCA is applied to different types of malignant tumors, it will express its molecular mechanism in different ways. For example, LCA has been shown to restrain cell proliferation by reducing DNA synthesis in cancer cells in a dose-dependent manner. Furthermore, supplementation with LCA may be conducive to counteracting the side effects of cisplatin therapy in cancer patients (Lee et al., 2008). In a study of gastric cancer, LCA was shown to affect gastric cancer cell viability by blocking cell cycle progression and inducing apoptosis. In addition, LCA induces autophagy through the mTOR pathway in prostate cancer cells (Yo et al., 2009). Furthermore, LCA was determined to induce apoptosis via the FasL signaling pathway (Kim et al., 2014). Nevertheless, there have been no reports on the antioxidant and anticancer activity of LCA alone in hepatocytes, specifically HepG2 cells. In view of this, we explored the antioxidant activity of the natural compound LCA, and based on this antioxidant activity, we also investigated the potential anticancer role of LCA.

2. Materials and methods

2.1. Chemical reagents

LCA was purchased from ChengDu Must Bio-Technology Co. Ltd. Dulbecco's modified Eagle's medium (DMEM), acquired from Corning (USA), and fetal bovine serum (FBS), acquired from Invitrogen-Gibco (USA), were used to culture hepatospheroid (HepG2) cells and L-02 cells. Anti-c-Jun N-terminal kinase (JNK), anti-phosphorylated JNK (p-JNK), anti-p38, anti-phosphorylated p38 (p-p38), anti-extracellular signal-regulated kinase 1/2 (ERK1/2), and anti-phosphorylated ERK1/2 (p-ERK1/2) were purchased from Cell Signaling Technology; anti-superoxide dismutase 1 (SOD1) and anti-glutathione peroxidase 1 (GPx1) were purchased from Abcam (UK). The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was purchased from Sigma (USA).

2.2. Cell culture

The L-02 and HepG2 cells were provided by the Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were cultured in DMEM supplemented with 100 units/mL penicillin, 10% FBS and 100 μg/mL streptomycin at 37 °C in 5.0% CO_2.

2.3. Antioxidant activity

2.3.1. CAA assay

A cellular antioxidant activity (CAA) assay is a more biologically relevant approach to measuring antioxidant activity. HepG2 cells were seeded at a density of 6×10^4/well into a 96-well plate in 100 μL of growth medium for 24 h. Then, the culture medium was removed, and the wells were washed with PBS. Accordingly, triplicate wells were treated for 1 h with 100 μL of LCA and 25 μM DCFH-DA dissolved in treatment medium and incubated in 5.0% CO_2 at 37 °C. In the uniform concentration gradients, some of the cells were washed with PBS, and the others were not. Then, 600 μM ABAP in 100 μL of HBSS was applied to the cells. The emission at 580 nm was measured with excitation at 485 nm every 5 min for 1 h with a microplate reader (Bio-Tek, USA). Control wells contained cells treated with DCFH-DA and oxidant; blank wells contained cells treated with dye and HBSS without oxidant.

Quantification of CAA. After subtracting the blank from the fluorescence readings, the area under the curve of fluorescence versus time was integrated to calculate the CAA value at each concentration of LCA:

$$\text{CAAunit} = 100 - \left(\int \text{SA} / \int \text{CA}\right) \times 100$$

where ∫ SA is the integrated area under the sample fluorescence versus time curve and ∫ CA is the integrated area from the control curve. From these data, we can calculate the EC_{50} of LCA.

2.3.2. Western blotting for SOD, CAT and GPx1 protein analyses

Cells (5×10^6 cells per well) were placed in culture dishes. After treatment with LCA for 24 h, cells were gathered, lysed using cell lysis buffer (Cell Signaling Technology) containing phosphatase and protease inhibitor cocktails, and incubated for more than 0.5 h at 4 °C. Lysates were centrifuged at 14,000× *g* for 10 min at 4 °C. The supernatant was used as the cytosolic fraction. Cellular proteins were extracted and quantified using a BCA kit (KeyGEN Biotech, China). Western blotting was implemented using 60 μg of the protein sample, and then, all of the membranes were incubated with the following primary antibodies: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and β-actin, which served as an internal control (1:500), at 4 °C for 12 h. Then, all membranes were incubated with anti-rabbit and anti-mouse antibodies at ambient temperature for 1 h. To obtain precise results, the immunoreactive bands were visualized using Image J software and exposed on radiographic film.

2.4. Antitumor activity

2.4.1. Cell viability assay

The MTT assay was based on the protocol described for the first time by Mosmann et al., 1983. Cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which is based on the conversion of MTT to formazan crystals by mitochondrial dehydrogenases. Briefly, cells (5×10^4 cells/mL) were seeded in triplicate in 96-well plates. Following overnight incubation at 37 °C for 24 h and 48 h, separately. The cells were treated with different concentrations of LCA for the indicated period of time. Finally, after incubation with various concentrations of LCA for 24 h or 48 h, 20 μL of MTT solution (5 mg/mL stock) was added to the cells, and then, the cells were incubated at 37 °C for 1 h. The number of viable cells is directly proportional to the production of formazan, and the result was read at 490 nm as the absorbance value using a microplate reader (Bio-Tek, USA) (de Sousa et al., 2004; Lu et al., 2015).

2.4.2. Evaluation of apoptosis

By using the DNA-specific fluorescent dye, DAPI (Vectashield; Vector Laboratories, Burlingame, CA, USA), we detected how the morphology of apoptotic cells changed in the nucleus, as previously described. The HepG2 cells were seeded on glass coverslips and treated with LCA for 24 h. The cells in each well were fixed with 500 μL of DAPI at room temperature for 15 min and washed with methanol. Then, the nuclear morphology of cells was observed with fluorescence microscopy (Nikon, Japan).

2.4.3. Western blotting for p-ERK, p-JNK and p-p38 protein analyses

HepG2 cells were grown in 6-well plates (6.25×10^4 cells/mL) and cultured for 24 h. Then, cellular proteins, which were extracted from HepG2 cells treated with LCA, were subjected to western blotting analysis. The primary antibodies used in the experiment were p-ERK, p-JNK and p-p38, and the secondary antibodies were anti-mouse and anti-rabbit. Bands were visualized with a chemiluminescence detector (DNR, Kiryat Anavim, Israel).

2.5. Statistical analysis

All data are presented as the mean ± standard deviation of at least three independent trials. Statistical analysis was conducted using SPSS 11.5 statistical software. P values of < 0.05 were considered statistically significant.