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Phenolcarboxylic acids from medicinal herbs exert anticancer effects through disruption of COX-2 activity

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ABSTRACT

Integrated research of herbs and formulas characterized by functions of promoting blood circulation and removing blood stasis is one of the most active fields in traditional Chinese medicine. This paper strives to demonstrate the roles of a homologous series of phenolcarboxylic acids from these medicinal herbs in cancer treatment *via* targeting cyclooxygenase-2 (COX-2), a well-recognized mediator in tumorigenesis. We selected thirteen typical phenolcarboxylic acids (benzoic acid derivatives, cinnamic acid derivatives and their dehydration-condensation products), and found gallic acid, caffeic acid, danshensu, rosmarinic acid and salvianolic acid B showed 50% inhibitory effects on hCOX-2 activity and A549 cells proliferation. 2D-quantitative method was introduced to describe the potential structural features that contributed to certain bioactivities. We also found these compounds underwent responsible hydrogen bonding to Arg120 and Ser353 in COX-2 active site residues. We further extensively focused on danshensu [D-(+)-β-(3,4-dihydoxy-phenylalanine)] or DSS, which exerted COX-2 dependent anticancer manner. Both genetic and pharmacological inhibition of COX-2 could enhance the ability of DSS inhibiting A549 cells growth. Additionally, COX-2/PGE_2/ERK signaling axis was essential for the anticancer effect of DSS. Furthermore, combined treatment with DSS and celecoxib could produce stronger anticancer effects in experimental lung metastasis of A549 cells *in vivo*. All these findings indicated that phenolcarboxylic acids might possess anticancer effects through jointly targeting COX-2 activity in cancer cells and provided strong evidence in cancer prevention and therapy for the herbs characterized by blood-activating and stasis-resolving functions in clinic.

Introduction

Supportive care of cancer patients with traditional Chinese medicine (TCM) has been an area of great interest, but the inherent challenges are embedded in the complexity of bioactive substances. Surprisingly, TCM practitioners have discovered abundant homologous compounds in medicinal plants, which may jointly improve the clinical efficacy mediated by Chinese medicinal prescriptions. Herbs characterized by blood-activating and stasis-resolving functions (Liao 2000) has displayed distinguished effects in treatment of cardiovascular diseases (CVDs) through anti-coagulation, anti-thrombosis and other improving hemorheological events (Gao et al. 2012; Liu et al. 2012). Noticeably, the effectiveness of the remedy has intrigued intensive investigation in patients with advanced cancers coupled with hypercoagulable and prothrombotic state (Lu and Li 2009; Ren et al. 2005; Tan et al. 2008). Nevertheless, the bioactive substances and underlying mechanisms linking the two

Abbreviations: TCM, traditional Chinese medicine; COX, cyclooxygenase; PGE2, prostaglandin E2; ERK, extracellular signal-regulated kinase; NSAIDs, non-steroidal anti-inflammatory drugs; CVDs, cardiovascular diseases; ELISA, enzyme linked immunosorbent assay; NSCLC, non-small cell lung cancer; SAR, structure and activity relationship; SA, salicylic acid; AA, anisic acid; PCA, protocatechuic acid; VA, vanillic acid; GA, gallic acid; SyrA, syringic acid; CA, cinnamic acid; CFA, caffeic acid; FA, ferulic acid; IFA, isoferulic acid; DSS, Danshensu; RA, rosmarinic acid; Sal B, salvianolic acid B; AA, arachidonic acid; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum.

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therapeutic means are still poorly understood. Here, we developed an approach called structure-based ligand similarity screening by referring to well-known positive compounds with definite drug targets, which could not only quickly discover bioactive substances in medical herbs in terms of ligand structure, but the pharmacological mechanisms underling therapeutic targets of unknown compounds could also be simply identified by experimental validation.

In this study, non-steroidal anti-inflammatory drugs (NSAIDs) displayed such reference compounds. The comprehensive epidemiological observations have demonstrated that daily use of NSAIDs or cyclooxygenase-2 (COX-2) selective inhibitors can reduce the risk of colorectal cancer (Arber et al. 2006; Chan et al. 2007; Clevers 2006; Langley et al. 2011). We observed that aspirin worked as the primary prevention for CVDs by not only inhibiting COX-1 activity in blocking platelet aggregation and thrombopoiesis, but also reducing cancer risk by impeding aberrant COX-2 activity and subsequent over-production of prostaglandins in carcinogenesis (Chan et al. 2007). Therefore, we proposed that cyclooxygenases might emerge as the primary pharmacological targets for blood-activating and stasis-resolving herbs both in CVDs and cancer treatments. We also noticed that overwhelming majority of NSAIDs were ascribed to phenolcarboxylic acids, such as salicylates (aspirin), propionic acids (flurbiprofen), phenylacetic acids (diclofenac), indoleacetic acids (indometaci), and pyrrolealkanoic acids (tolmetin) and so on (Dannhardt and Kiefer 2001), which formed the structure basis of inhibitory effects on COX. Therefore, we can define phenolcarboxylic acids from medical herbs sharing similar pharmacological actions with NSAIDs. Significantly, research evidence has revealed that blood-activating and stasis-resolving herbs comprise most abundant phenolcarboxylic acids closely related to their medical functions (Tang et al. 2008). Table 1 shows the basic information of the selected thirteen typical phenolcarboxylic acids and they were classified into three groups in terms of structure patterns: benzoic acid derivatives, cinnamic acid derivatives and their condensation products. Based on the structure–activity relationship (SAR), we postulated that these compounds might impede COX-2 activity in cancer therapies.

To illustrate the mechanisms underlying anticancer activities of phenolcarboxylic acids, the present study was investigated in non-small cell lung cancer (NSCLC), a leading systematic malignant disease in both incidence and mortality worldwide (Siegel et al. 2013). This work was aimed to clarify whether the altered COX-2 activity by these unique phenolcarboxylic acids was involved in the pharmacological actions and traditional functions in metastatic disease.

Materials and methods

Chemicals and bioreagents

Celecoxib (PubChem CID: 2662) was purchased from Nanjing DeBioChem Co., Ltd (Jiangsu, China) and dissolved in 10 mM stock in DMSO. Aspirin or acetylsalicylic acid (PubChem CID: 2244) were purchased from Sigma; Danshensu (PubChem CID: 439435) and Salvianolic acid B (PubChem CID: 6441188) were ordered from Xi'an Helin Biological Engineering Co., Ltd (Xi'an, China); Salicylic acid (PubChem CID: 338), Ferulic acid (PubChem CID: 445858), Caffeic acid (PubChem CID: 689043), Protocatechuic acid (PubChem CID: 72), Anisic acid (PubChem CID: 7478), Gallic acid (PubChem CID: 370), Vanillic acid (PubChem CID: 8468), Syringic acid (PubChem CID: 10742), Cinnamic acid (PubChem CID: 444539), Isoferulic acid (PubChem CID: 736186), and Rosmarinic acid (PubChem CID: 5281792) were ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Danshensu and Salvianolic acid B were dissolved in 100 mM stock in PBS and the remaining compounds were in DMSO before use. Arachidonic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl) and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The compounds were over 90% purity based on HPLC analysis.

DPPH radical scavenging activity

The antioxidant property of phenolcarboxylic acids was determined by DPPH method. Briefly, the drug solution and alcoholic DPPH solution (0.4 mM) were added into the triplicate wells of 96 well plate and co-incubated for 30 min. The absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a positive control. The DPPH-scavenging activity (%) = (absorbance of vehicle control − absorbance of observation group) × 100/absorbance of vehicle control.

Cell line and cell culture

A549 human lung carcinoma cell line was purchased from the Chinese Academy of Sciences Cell Bank of Type Culture Collection (CBTCCCAS, Shanghai, China) and authenticated by analysis of the short tandem repeat DNA profile. Cells were maintained in RPMI-1640 complete medium.

Cell viability assay

Cell viability was determined by the MTT reagent as previously described (Zhang et al. 2010). IC_{50} values were calculated by the method of Probit using PASW Statistics 18 software.

Virtual screening assay

A virtual screening of potential inhibitors against COX-2 was performed using Molegro Virtual Docker software (MDV; http://www.molegro.com), its algorithm called MolDock showed a higher docking accuracy of 87% compared to other docking products in the market (Thomsen and Christensen 2006). Mol2 formats of various compounds were available in ZINC database (http://zinc.docking.org). The 3D X-ray structure of COX-2 and co-crystallized ligand SC-558 (PDB; http://www.rcsb.org; PDB code: 6COX) were selected as a receptor model. Before the docking program, the flexibility of each compound and search space as a sphere of 10 Å around the center of mass of SC-558 were configured. The docking protocol was validated on re-docking of SC-558 with COX-2.

Purified hCOXs activity assay

The COX-1 and COX-2 enzymatic inhibitory rates were performed by incubation of various compounds with human recombinant COX-1 and COX-2 (Cayman Chemical, Ann Arbor, MI). The reaction rates were the slope value by plotting the kinetic change of absorbance within 5 min. The inhibitory rate = (reaction rate of vehicle control − reaction rate of observation group)/reaction rate of vehicle control.

Quantitative structure–activity relationship (QSAR) study

A quantitative structure activity relationship study was performed on a series of phenolcarboxylic acid derivatives possessing COX-2 inhibitory activity for establishing quantitative relationship between biological activity and their structural properties. MOE software package (Molecular Operating Environment, Chemical Computing Group: http://www.chemcomp.com/) was employed

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