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Functionalized magnetic nanoparticles coupled with mass spectrometry for screening and identification of cyclooxygenase-1 inhibitors from natural products

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ABSTRACT

Development of simple and effective methods for high-throughput, high-fidelity screening and identification of cyclooxygenase-1 (COX-1) inhibitors from natural products are important for drug discovery to treat inflammation and carcinogenesis. Here, we developed a new screening assay based on cyclooxygenase-1 (COX-1) functionalized magnetic nanoparticles (i.e. Fe_3O_4@SiO_2–COX-1) for solid phase ligand fishing, and then mass spectrometry (MS) was applied for structural identification. Incubation conditions were optimized. High specificity for isolating COX-1 inhibitors was achieved by testing positive control, indomethacin, with active and inactive COX-1. Moreover, high stability of immobilized COX-1 (remained 95.3% after ten consecutive cycles) allows the analysis reproducible. When applied to turmeric extract, four curcuminoids (i.e. curcumin, demethoxycurcumin, bisdemethoxycurcumin, and 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(1*E*,6*E*)-1,6-heptadiene-3,5-dione), difficult to be distinguished from original MS spectrum of turmeric extract, were isolated as main COX-1 inhibitors. Their structures were characterized based on their accurate molecular weight and diagnostic fragment ions. The results indicated that the proposed method was a simple, robust and reproducible approach for the discovery of COX-1 inhibitors from complex matrixes.

1. Introduction

Enzymes are attractive drug targets, and enzyme inhibitors represent almost half the drugs used in clinical today [1]. Therefore, discovery of new enzyme inhibitors has been one of the major interests and challenges in drug discovery and development process [2]. Historically, majority of new drugs were generated from natural products or compounds derived from natural products [3]. From 1981 to 2010, 50% of all the marketed-new chemical entities were shown to be of natural origin [4]. Undoubtedly, natural products continue to play a highly significant role in the discovery of drug leads.

Natural products embody hundreds or even thousands of secondary metabolites, but a few of them are responsible for the pharmacological activity. The key step in natural products research is to develop high throughput, high fidelity methods for discovery of bioactive components. Moreover, the process of modernization and globalization of natural products necessitate the biochemical profiles analysis for quality control purpose. To address these requirements, biofingerprint chromatogram based on ligand fishing has been proposed to provide unique information on the multiple bioactive compounds direct interaction with drug targets (i.e. protein, enzyme and receptor). Ligand fishing assay has been considered as the most convenient and efficient technology with high-selectivity and high-throughput in early stage bioactive components discovery, which include, but are not limited to, centrifugation [6], ultrafiltration [7–9], equilibrium dialysis [10], microdialysis [11], magnetic solid phase fishing [7,12], and surface plasmon resonance [13,14]. Immobilized drug targets on solid surface are more robust and resistant to environmental changes than solution phase drug targets [7,15]. At the same time,

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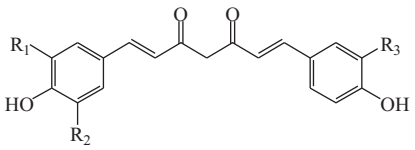

$R_1 = R_3 = OCH_3$, $R_2 = H$; Curcumin (**1**)

$R_1 = R_2 = H$, $R_3 = OCH_3$; Demethoxycurcumin (**2**)

$R_1 = R_2 = R_3 = H$; Bisdemethoxycurcumin (**3**)

$R_1 = R_2 = R_3 = OCH_3$; 1-(4-Hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(1*E*,6*E*)-1,6-heptadiene-3,5-dione (**4**)

Fig. 1. Chemical structures of four investigated curcuminoids.

magnetic nanoparticles could be separated from solution conveniently. Therefore, ligand fishing based on functionalized magnetic nanoparticles has been proved to be an exciting method to screen ligands from natural products [7,12].

COX-1 is known as a housekeeping enzyme constitutively expressed in almost all the mammalian tissues. The prostaglandins produced from arachidonic acid transformation through the participation of COX-1 play an important role in inflammation and carcinogenesis [16]. Importantly, platelet COX-1 is the target of one of the most efficacious antithrombotic agents used for prevention of vascular occlusive events (i.e. aspirin) [17], which thereby provides the rationale for the development of COX-1 inhibitors. Many reports have focused on the detection of COX-1 inhibitory activity of commercial isolated compounds from natural products or synthesized compounds [17–20]. However, much less attention has been paid to develop facile screening assay to discover COX-1inhibitors from natural products.

Turmeric is dried powder from rhizomes of *Curcuma longa* L. (Zingiberaceae), which has been used as a traditional medicine for its various pharmacological activities such as anti-inflammatory, antiviral, antioxidant, anti-infectious activities, anti-parasitic infection, anti-mutagenic effect, and anticancer [21,22]. The underlying mechanisms of these effects involve the recognition of various molecular targets, such as enzymes and protein kinases. Recent studies showed that curcuminoids, the major yellow pigment and active components of turmeric [23], had significantly higher inhibitory effects on the peroxidase activity of COX-1 than COX-2 [24]. However, no reports systematically analyzed COX-1 inhibitors in turmeric.

Herein, we report proof of principle for the first time of integration of COX-1 functionalized magnetic nanoparticles and direct infusion MS for facile, specific screening and identification of COX-1 inhibitors from complex natural products. Four curcuminoids (i.e. curcumin [1], demethoxycurcumin [2], bisdemethoxycurcumin [3], and 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(1*E*,6*E*)-1,6-heptadiene-3,5-dione [4], Fig. 1) with COX-1inhibitory activity were isolated from turmeric.

2. Experimental

2.1. Chemicals and reagents

Ovine COX-1, arachidonic acid, prostaglandin E_2 (PGE_2), [d_4]-PGE_2, co-factors L-epinephrine and hematin, 3-aminopropyltrimethoxysilane (APTMS), tetraethyl orthosilicate, and glutaraldehyde (25%, w/v aqueous solution) were acquired from Sigma–Aldrich Chemicals (St. Louis, MO, USA). The HPLC grade acetonitrile and methanol were bought from Tedia Company, Inc. (Ohio, USA). Ultrapure water (18.2 MΩ) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Buffer solution used all over the experiments was 10 mM ammonium acetate buffer solution with pH at 7.4. All of other chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Reference compounds, curcumin (**1**), demethoxycurcumin (**2**), bisdemethoxycurcumin (**3**), and positive control sample, indomethacin, with purities over 99% were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

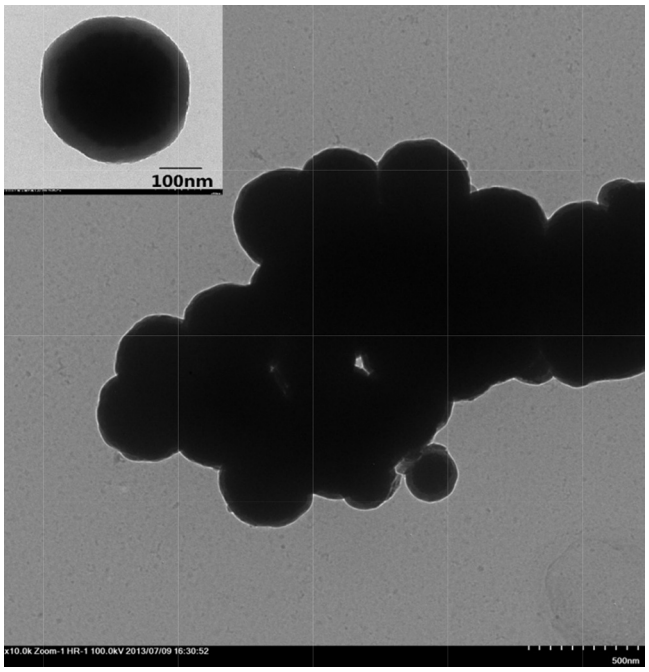

Fig. 2. TEM image of the synthesized Fe_3O_4@SiO_2–COX-1 nanoparticles.

2.2. Preparation of turmeric extraction

Turmeric was purchased from local drugstore in Changsha, which was identified as *C. longa* L. by one of the authors, Prof. Mijun Peng.

Approximate 20 g of turmeric was powdered and extracted with 300 ml of 75% (v/v) ethanol three times, each for 3 h, and the filtrates were concentrated on a rotary evaporator (Shanghai Yarong Biochemical Instrument Factory, Shanghai, China) under reduced pressure at 40 °C to yield dried residue (3.1 g). A stock solution (3 mg/ml) of the residue was then stored at 4 °C for further experiments.

2.3. COX-1 inhibition assay

COX-1 inhibitory assay was performed according to a previously described PGE_2 (a stable oxidation product resulting from COX-1 oxidation of arachidonic acid) detection method [25]. In brief, 2 μl of hematin (100 μM) mixed with 10 μl of L-epinephrine (40 mM) and made up with buffer solution to a final volume of 140 μl. Then 20 μl of COX-1 (0.1 μg) was added and incubated at 25 °C for 2 min. After that, 20 μl of different concentrations of COX-1 ligands were added and preincubated at 37 °C for 10 min. The COX-1 inhibition reaction was initiated by adding 20 μl of arachidonic acid (50 μM) and terminated by adding 20 μl of HCl (2.0 M). The concentration of product PGE_2 was detected by HPLC–MS/MS method by using [d_4]-PGE_2 as surrogate standard. The inhibitory activity was determined by comparing the amount of PGE_2 produced with that of