Journal of Pharmaceutical and Biomedical Analysis 151 (2018) 42–48

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Rapid screening of brain-penetrable antioxidants from natural products by blood-brain barrier specific permeability assay combined with DPPH recognition

Junfeng Zhu[a,b], Xiaojiao Yi[a], Jinghui Zhang[a], Shuqing Chen[a], Yongjiang Wu[a,*]

[a] College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, PR China
[b] Laboratory of Clinical Pharmacy, Zhejiang Cancer Hospital, Hangzhou 310022, PR China

ARTICLE INFO

Article history:
Received 2 November 2017
Received in revised form
17 December 2017
Accepted 26 December 2017
Available online 28 December 2017

Keywords:
Blood-brain barrier permeability
Antioxidant
PAMPA
DPPH
2,6,4′-trihydroxy-4-methoxybenzophenon

ABSTRACT

Screening brain-penetrable antioxidants from natural products is a promising way for neuroprotective drug discovery. However, there is no screening methodology enables simultaneous investigation of antioxidant activity and blood-brain barrier (BBB) permeability of compounds from complex samples. Here we propose a novel strategy by combining BBB specific parallel artificial membrane permeability assay with 1,1-diphenyl-2-picrylhydrazyl recognition (BBB-PAMPA–DPPH) to achieve rapid multicomponent screening. First, BBB specific artificial membrane was constructed to separate the compounds with high BBB permeability in herbal extracts. The antioxidant activity of the isolated compounds could be optically recognized through the bleaching of the purple-colored DPPH. By off-line combined HPLC–UV/Q-TOF-MS analysis, the exact BBB-penetrable compounds responsible for the antioxidant activity could be rapidly screened. With this approach, compound 2,6,4′-trihydroxy-4-methoxybenzophenon in Rhizoma Anemarrhena was found to be an antioxidant with very high BBB permeability, which could also be detected in rat plasma and brain tissue after oral administration. Our findings suggested the BBB-PAMPA–DPPH method could be a powerful tool for neuroprotective drug discovery from natural products.

1. Introduction

Neurodegenerative diseases (NDs), such as Alzheimer's, Parkinson's, and Huntington's disease, are disorders in which the nervous system progressively and irreversibly deteriorates. The etiology of NDs are quite elusive. Oxidative stress, inflammation, mitochondrial dysfunction, apoptosis and many other events are involved in the pathogenesis of NDs [1]. However, oxidative stress resulting from the unbalance between production and detoxification of reactive oxygen species is believed to be the early and fundamental mechanism [2–6]. It has been proposed that oxidative stress in the central nervous system (CNS) could injury biomolecules including DNA, proteins and lipids, leading to cellular damage and subsequent cell death [7]. Thus, therapeutic strategies aimed at the prevention of free radicals are widely-recognized and considerable efforts are currently devoted to the development of antioxidants as neuroprotective drugs [8–10].

Natural products possess a high chemical scaffold diversity, which have proven historically to be rich sources for multifarious antioxidants like vitamins, flavones and tannins [11,12]. However, the therapeutic potential of most of these compounds is shadowed by their poor permeability to cross the blood-brain barrier (BBB) [13]. It has been reported that almost all macromolecular drugs and over 98% of small molecule drugs could not pass the BBB [14]. Along with having suitable antioxidant activity, BBB permeability should be taken as a key factor in neuroprotective drug discovery [15]. Unfortunately, there is no screening methodology enables simul-

Abbreviations: BBB, blood-brain barrier; PAMPA, parallel artificial membrane permeability assay; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ND, neurodegenerative disease; CNS, central nervous system; RA, Rhizoma Anemarrhena; ZM, 2,6,4′-trihydroxy-4-methoxybenzophenon; VC, ascorbic acid; VE, α-tocopherol; BHT, butyrate hydroxyltoluene; SE, sesamol; PBL, porcine polar brain lipid; PBS, phosphate buffer solution; Pe, effective permeability; HPLC, high performance liquid chromatography; UV, ultraviolet detector; Q-TOF-MS, quadrupole time-of-flight mass spectrometer; RSA, radical scavenging activity.

* Corresponding author.
E-mail address: yjwu@zju.edu.cn (Y. Wu).

https://doi.org/10.1016/j.jpba.2017.12.055
0731-7085/

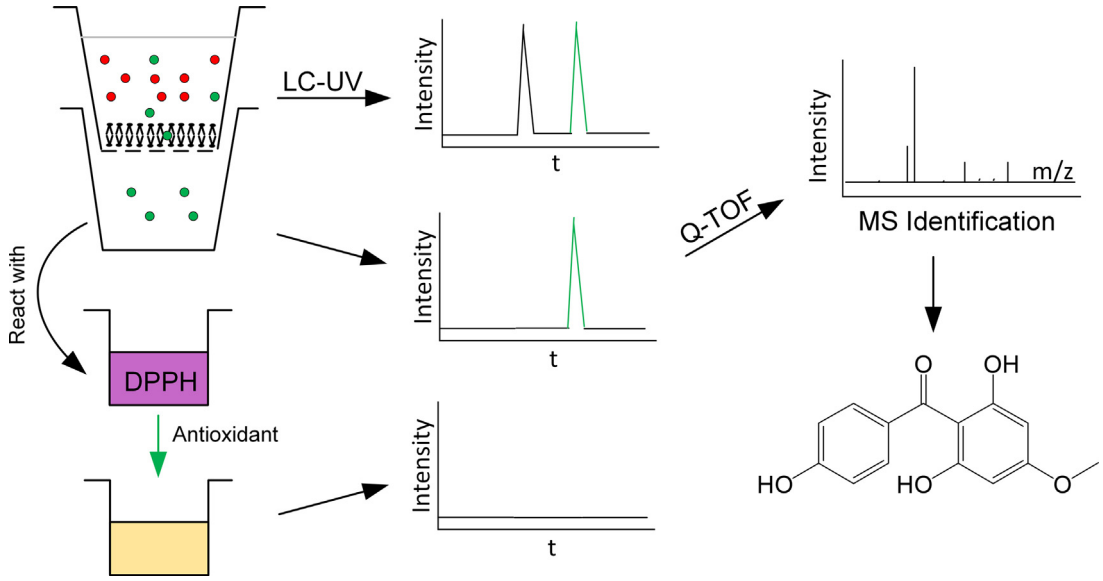

Fig. 1. The schematic diagram of BBB-PAMPA–DPPH assay.

taneous investigation of antioxidant activity and BBB permeability of compounds from natural products.

In vivo BBB models are costly, low-throughput and impractical for the investigation of complex mixtures through the BBB. Previously, *in vitro* BBB-specific parallel artificial membrane permeability assay (BBB-PAMPA) was developed to predict the BBB penetration of commercial drugs [16]. In recent years, BBB-PAMPA was optimized by Balogh et al. and successfully applied in the screening of brain-penetrable compounds from natural products [17]. This technique uses an artificial lipid membrane as a physicochemical filter to separate BBB-penetrable compounds from complex mixtures. It allows to model simultaneously the rate of transcellular passive diffusion of each compound in complex mixtures across the BBB by determining the effective permeability (Pe, cm/s). For example, brain penetrability of the constituents in *Ginkgo biloba* L. extracts were characterized using this method [18].

With the aim of developing innovative screening methods enabling a rapid and efficient identification of antioxidants with high brain penetration propensity, here we propose a simple protocol based on BBB-PAMPA combined with 1,1-diphenyl-2-picrylhydrazyl recognition (BBB-PAMPA–DPPH). The schematic diagram of the proposed approach is shown in Fig. 1. First, BBB specific artificial membrane was constructed to separate the compounds with high BBB permeability in herbal extracts. The antioxidant activity of the isolated compounds could be optically recognized through the bleaching of the purple-colored DPPH. By off-line combined HPLC–UV/Q-TOF-MS analysis, the exact BBB-penetrable compounds responsible for the antioxidant activity could be rapidly screened [19]. The proposed method was applied in the high-throughput screening of potential brain-penetrable antioxidants for the first time. Compound 2,6,4′-trihydroxy-4-methoxybenzophenon (ZM) in Rhizoma Anemarrhena (RA) was found to be an active substance.

2. Experimental

2.1. Materials and reagents

Medicinal plants were purchased from Anhui Yuanhetang Pharmaceutical Co., Ltd. (Anhui, China). Species of these plants were listed in the Supplementary Materials (Table S1). The porcine polar brain lipid (PBL) (Catalog No. 141101P) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol, *n*-dodecane, ascorbic acid (VC), α-tocopherol (VE), butyrate hydroxyltoluene (BHT) and sesamol (SE) were purchased from Sigma-Aldrich (St. Louis, MO). Reference standard of 2,6,4′-trihydroxy-4-methoxybenzophenon (ZM, purity >98%) was purchased from BioBioPha Co., Ltd. (Yunnan, China). DPPH (purity of 98%) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). HPLC-grade methanol and acetonitrile were purchased from J & K scientific Ltd. (Shanghai, China). All other chemicals and solvents were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water was purified using a Milli-Q water-purification system from Millipore (Bedford, MA, USA).

The 96-well donor plate (Catalog No. MAIPN4550, MultiScreen-IP filter plate, PVDF membrane, 0.45 μm) was purchased from Millipore (Bedford, MA, USA). The 96-well acceptor plate (PP, 0.5 mL) was purchased from Agilent (Agilent Technologies, USA).

2.2. Sample preparations

A total of 46 kinds of medicinal plants were extracted in the same way as previously described [20]. Briefly, decoction pieces of medicinal plant (100 g) were extracted with 70% ethanol twice under reflux. Combined ethanol solution was concentrated under reduced pressure to give a crude extract. After defatted with petroleum ether, the crude extract was extracted with ethyl acetate (1:1, v/v) for three times. The ethyl acetate phase was evaporated to dryness in vacuum to obtain the final extract. Stock solutions of the plant extracts were prepared uniformly in methanol at 50 mg/mL, which was diluted 1:10 in phosphate buffer solution (PBS, 10 mM, pH 7.4) and centrifuged at 13,000 rpm for 10 min before subjected to the BBB-PAMPA–DPPH experiments.

The stock solutions of VC, VE, BHT, SE and ZM were prepared in methanol at a concentration of 10 mM, respectively. Stock solutions were diluted by methanol in the DPPH radical scavenging assay. While in the BBB-PAMPA–DPPH experiments, the stock solutions were diluted by PBS and the concentration of methanol in the samples never exceeded 20% (v/v).

2.3. BBB-PAMPA–DPPH for screening of active plant extracts

BBB specific artificial membrane was constructed to separate the compounds with high brain penetration propensity from the plant