Fitoterapia 106 (2015) 110–114

Contents lists available at ScienceDirect

Fitoterapia

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

Synthesis, *in silico* and *in vivo* blood brain barrier permeability of ginkgolide B cinnamate

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ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form 22 August 2015
Accepted 25 August 2015
Available online 29 August 2015

Keywords:
Ginkgolide B
Cinnamic acid
Blood brain barrier
Brain-targeting
LC–MS
Theoretical simulation

ABSTRACT

Ginkgolide B, one of the important components of *Ginkgo biloba* extracts, has been revealed to exhibit great potential in therapy of cerebrovascular diseases. However the lack of permeability greatly limited it from further clinical application. Based on the prediction model for blood brain barrier (BBB) permeation, herein a potential brain-targeting analog ginkgolide B cinnamate (GBC) was successfully synthesized and characterized. After intravenous administration of GBC or GB, liquid chromatography tandem mass spectrometry (LC–MS/MS) was conducted to determine the analog in rat plasma and brain. The results showed that GBC had a significant increase in BBB permeability. A significant 1.61-times increase in half-life was observed for GBC and the drug targeting index (DTI) value was calculated to be 9.91. The experiment results matched well with the predicted one, which revealed that BBB permeability prediction model combined with *in vivo* study could be used as a quick, feasible and efficient tool for brain-targeting drug design.

1. Introduction

Ginkgo biloba, one of the oldest plants on earth, has been extensively used for pharmaceutical and medical purposes in China for several hundred years. Nowadays, *G. biloba* extracts have become one of the most common and best explored herbal medicinal products [1,2] since the pioneering work by Furukawa [3], Maruyama [4] and Weinges [5] who demonstrated that ginkgolides A, B, C, M and J were the main efficient components. As an important component of *G. biloba* extracts, ginkgolide B (GB) was revealed to be the best antagonist of platelet activating factors [6] and extensive studies were already made for GB and its analogs [7,8]. Besides this GB also has a great potential in the therapy of cardiovascular and cerebrovascular diseases such as preventing Alzheimer's disease [9], neuron protection [10], enhancing the expression of nerve growth [11], and reducing neuronal cell apoptosis [12].

Ischemic cerebrovascular disease is a kind of common and frequent disease with high disability and mortality rate. It is extensively accepted that this is one of the severe diseases that threaten the health and affect the living quality of the elderly and old people. Although GB has presented a significant therapeutic potential in cardiovascular and cerebrovascular diseases [13,14], the presence of the unique blood brain barrier (BBB) inhibits its distribution in the central nervous system (CNS) [15]. As reported by Fang et al., GB does have optimal brain penetration [16]. However the penetration is so limited that GB can't be largely delivered into the brain to reach an effective therapeutic concentration. In order to develop GB as a potential drug for ischemic cerebrovascular diseases, there is a need to increase the dosage or enhance the delivery across the BBB. Obviously increasing the permeability is a safe and feasible strategy. Various attempts [17] have already been tried to enhance the delivery of various CNS drugs across the BBB, such as disruption of BBB [18–20], adsorptive-mediated transcytosis [21,22], carrier-mediated transport [23], receptor-mediated transcytosis [24–27], nanoparticles [28,29], prodrugs [30,31], inhibition of efflux pumps [32], and intranasal drug delivery [33,34]. Among these efforts, modification of drugs has been proved to be a safe, efficient and feasible strategy for enhancing delivery. The delivery of GB to the brain across the BBB could also be benefited by chemical modification of the molecule. Study on brain-targeting ability of GB analogs not only paves the way for design and synthesis of GB-based drugs, but also favors the therapy for central nervous system diseases in the future.

As illustrated in the literature [30,31], modification of organic compounds with specific functional groups could greatly affect their permeabilities. On the other hand natural products also have some pharmacoactivities with no or low toxicity, which might enhance the therapy effect. Therefore modification of GB with a natural product might be an effective strategy to enhance the delivery of GB across the

Abbreviations: BBB, blood brain barrier; CNS, central nervous system; DTI, drug targeting index; GB, ginkgolide B; LC–MS/MS, liquid chromatography tandem mass spectrometry; GBC, ginkgolide B cinnamate.

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http://dx.doi.org/10.1016/j.fitote.2015.08.012
0367-326X/

BBB and its therapy effect. To the best of our knowledge, there are few GB analogs synthesized like this. Wu et al. has reported an analog synthesized by esterification of GB with nicotinic acid [35]. The drug-targeting index of the GB analog was calculated to be 7.45 and area under curve (AUC) value of GB decomposed from the analog was 2.45-fold higher than that of GB in the brain. Cinnamic acid is a naturally aromatic fatty acid with low toxicity in human exposure [36]. In recent years, cinnamic acid derivatives have attracted much attention due to their anticancer, antioxidant, and antimicrobial properties [37]. A combination of GB and cinnamic acid not only might enhance the permeability, but also would provide a combined-modality therapy and reduce the risk of modified moieties.

Based on the prediction model for BBB permeation established by our group [38] and as a continuous study of GB in our lab [39–41], we report here the synthesis of the analog of ginkgolide B cinnamate (GBC) according to the lipid characteristics of the BBB and computational simulation. The tissue distribution and pharmacokinetics of the analog were studied *in vivo* compared with that of GB.

2. Materials and methods

2.1. Materials

Ginkgolide B (GB) was obtained with purity of more than 95%. The purity of standard preparation of ginkgolide B and bilobalide (BB) was >99.9%. Cinnamic acid is of analytical grade with purity of >99.5%. All the other reagents are of analytical grade and used without further purification. Water was purified by the Milli-Q system.

2.2. Characterization

^{1}H nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectra were recorded on an Avance 400 spectrometer (Bruker Corporation, Switzerland). Mass spectroscopy (MS) was obtained with an API 3200™ triple quadrupole mass spectrometer (Applied Biosystems, USA) equipped with an electrospray ionization (ESI) source. The Fourier transform infrared (FT-IR) spectrum was conducted using Nicolet 5700 FTIR spectrophotometer (Thermo Electron Corporation, USA). The LC–MS/MS method was established for the simultaneous determination of GB and GBC in plasma and brain homogenate. The 1100 series HPLC system (Agilent, USA) was used. The mobile phase was composed of 10 mM ammonium acetate and methanol (15:85, v/v), and its flow rate was 0.8 ml/min. The temperature of the column was kept at 40 °C. The API 3200 triple quadrupole tandem mass spectrometer with an ESI interface was operated in negative ionization mode. Analyst 1.3.2 software was used for instrument control and data acquisition and processing. The operation conditions were described as follows: ion spray voltage (ISV) −4500 V, heater gas temperature (TEM) 500 °C, curtain gas pressure (CUR) 15 psi, nebulizer gas pressure (GAS 1) 40 psi, heater gas pressure (GAS 2) 40 psi and collision gas pressure (CAD) 5 psi. Multiple reaction monitoring (MRM) mode was used for quantification, with ion transitions of m/z 423.6 → m/z 367.2, m/z 553.6 → m/z 146.9 and m/z 325.2 → m/z 163.3 for GB, GBC and BB (internal standard, IS), respectively.

2.3. General procedure for the synthesis of GBC

A mixture of cinnamic acid (96 mg, 0.65 mmol) and ginkgolide B (212 mg, 0.5 mmol) was completely dissolved in acetonitrile (15 mL) with the aid of sonication and cooled to 0 °C. Then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 115 mg, 0.65 mmol) and 4-dimethylamino pyridine (DMAP, 15 mg, 0.1 mmol) was added. After stirring at 0 °C for 1 h, the reaction mixture was stirred for another 10 h at room temperature. When the reaction time was over, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in 20 mL of ethyl acetate. After being washed by 5% aq. $NaHCO_3$ and saturated NaCl solution respectively, the organic phase was dried by anhydrous sodium sulfate. The crude product was then purified on a silica gel column with petroleum ether/acetone (v/v, 2:1) as the eluent to obtain the product.

Ginkgolide B cinnamate: white amorphous solid; yield 58.5%; purity >98%; FT-IR (cm − 1) νmax 3467.1 2961.5 2873.7 1786.3 1729.2 1633.2 1577.9 1450.7 1359.1 1336.7 1308.1 1279.7 1232.1 1203.3 1142.0 1116.3 1069.9 1053.9 988.7 963.6 924.5 885.0 767.1 683.0 496.7; ^{1}H NMR (400 MHz, CD3OD) δ7.99 (d, J = 13.6 Hz, C6H5CH = CH), 7.65–7.66 (t, 3H, C6H5), 7.44–7.45 (d, 2H, C6H5), 6.62 (d, J = 16.0 Hz, 1H, CH = CHCO), 6.31 (s, 2H, H-12), 6.27 (s, 1H, H-10α), 5.61 (s, 1H, H-6), 4.630 (d, J = 6.4 Hz, 1H, H-2), 4.33 (d, J = 6.4 Hz, 1H, H-1β), 3.08 (q, J = 7.0 Hz, 1H, H-14), 2.29 (d, J = 10.4 Hz, 1H, H-7β), 1.96–2.07 (m, 2H, H-8, H-7α), 1.26–1.33 (m, 3H, H-16), 1.07 (s, 9H, t-Bu); ^{13}C NMR (100 MHz, CD3OD)δ 6.97 (C-16), 27.86 (C-18, 19, 20), 31.72 (C-17), 36.70 (C-7), 41.92 (C-14), 49.16 (C-8), 67.46 (C-9), 69.32 (C-10), 72.40 (C-5), 74.04 (C-1), 79.01 (C-6), 83.15 (C-3), 94.74 (C-2), 99.97 (C-4), 110.74 (C-12), 115.60 (C-22), 128.23 (C-25,29), 128.69 (C-26,28), 130.68 (C-27), 134.11 (C-24), 147.77 (C-23), 164.85 (C-21), 169.01 (C-13), 171.08 (C-11), 176.98 (C-15); ESI MS (neg. ion mode) m/z 553.6 $[M–H]^-$; HR EIMS m/z calcd. for $[M–H]^-$ $C_{29}H_{29}O_{11}$ 553.5351, found 553.5425.

2.4. General procedure for the preparation of biological samples

The plasma and brain samples were handled with the same procedure. For example 100 μL of plasma samples and 20 μL of BB solution (dissolved in methanol, 0.1 μg/mL) were mixed in 5 mL of ethyl acetate. The mixture was vortexed for 5 min and centrifuged at 12,000 rpm for 10 min. After that the supernatant was evaporated under nitrogen gas at 50 °C. The residue was re-dissolved with 500 μL methanol solution (85% methanol for GB determination and 10% methanol for GBC). After being vortexed and centrifuged, 10 μL aliquot of the supernatant was injected into the liquid chromatography tandem mass spectrometry (LC–MS/MS) system for analysis.

Table 1
The parameters and predicted logBB[a] value of GB and GBC.

Compound	Volume	logP[b]	PSA[c]	P-gp (H)[d]	CSPB[e]	HBA[f]	HBB[g]	NRB[h]	Predicted logBB
GB	3.45	−0.09	1.50	0.27	0.19	0.63	0.46	2	−1.33
GBC	4.27	1.20	1.38	0.23	0.48	0.46	2.91	4	−0.37

[a] Defined as the logarithm of the brain/blood concentration ratios at steady-state.
[b] Octanol–water partition coefficients.
[c] Polar surface area.
[d] High affinity P-glycoprotein substrate probability.
[e] Plasma protein binding ratio.
[f] Abraham's hydrogen-bond acidity.
[g] Abraham's hydrogen-bond basicity.
[h] Number of rotatable bonds.