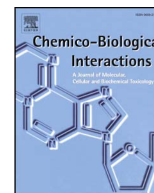
Chemico-Biological Interactions 278 (2017) 65–73

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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



Characterization of the inhibitory activity of natural tanshinones from *Salvia miltiorrhiza* roots on protein tyrosine phosphatase 1B

Da Hye Kim[a,1], Pradeep Paudel[a,1], Ting Yu[a], Thi Men Ngo[b], Jeong Ah Kim[b], Hyun Ah Jung[c,**], Takako Yokozawa[d], Jae Sue Choi[a,*]

[a] Department of Nutrition and Food Science, Pukyong National University, Busan 48513, Republic of Korea
[b] College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea
[c] Department of Food Science and Human Nutrition, Chonbuk National University, Jeonju 54896, Republic of Korea
[d] Graduate School Science and Engineering for Research, University of Toyama, Toyama 930-8555, Japan

A R T I C L E I N F O

Keywords:
Salvia miltiorrhiza
Lamiaceae
Tanshinone
Anti-diabetes
PTP1B
Molecular docking

A B S T R A C T

Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator that plays an important role in many signaling pathways, especially those associated with insulin resistance. In this study, we investigated the anti-diabetic potential of 12 natural tanshinones isolated from *Salvia miltiorrhiza* (*S. miltiorrhiza*) Bunge (Lamiaceae), deoxyneocryptotanshinone (**1**), grandifolia F (**2**), ferruginol (**3**), cryptotanshinone (**4**), tanshinone IIA (**5**), tanshinol B (**6**), tanshinone IIB (**7**), tanshinonal (**8**), methyl tanshinonate (**9**), 15,16-dihydrotanshinone I (**10**), tanshinone I (**11**), and dehydrodanshenol A (**12**) and evaluated their inhibitory activity against PTP1B. Tanshinones **4**, **6** and **12** exhibited potent PTP1B inhibitory activity with IC_{50} values of 5.5 ± 0.9, 4.7 ± 0.4 and 8.5 ± 0.5 μM, respectively. In addition, tanshinones **1–3**, **5** and **7–11** showed promising dose-dependent inhibition of PTP1B over IC_{50} values ranging from 18.6 to 254.8 μM. Enzyme kinetic analysis of PTP1B inhibition revealed that **4** and **6** were mixed -noncompetitive type inhibitors, whereas **12** was a classical-noncompetitive type inhibitor. Furthermore, **4**, **6** and **12** were docked with the PTP1B enzyme using molecular docking simulations (AutoDock 4.2) and exhibited negative binding energy (−6.4 to −8.7 kcal/mol), indicating high binding affinity to PTP1B active site residues. Structure-activity relationships (SAR) analysis revealed that structural modifications of ring A and furan or dihydrofuran ring D on the basic structure of tanshinones influenced their activity. Overall, results indicated that tanshinones from *S. miltiorrhiza* are potential anti-diabetic candidates that should be explored in the development of preventive and therapeutic modalities for the treatment of diabetes as well as diabetes-associated complications.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia and long-term disturbances in carbohydrate, lipid, and protein metabolism inducing chronic progressive dysfunction and failure of visual, nervous, renal, cardiac, and blood vascular systems; its incidence is increasing progressively in both developed and developing countries [1]. As of 2014, an estimated 387 million individuals worldwide had been diagnosed with DM and the prevalence is expected to rise to 592 million by 2035 [2]. In addition, the incidence of obesity, atherosclerosis, coronary heart disease, hypertension, and dyslipidemia is significantly higher in DM patients, with an earlier onset and faster progression compared with non-DM patients [1]. DM-induced vascular diseases including macrovascular and microvascular complications contribute to diminished quality of life and even death. Common risk factors for DM-related vascular disease include hyperglycemia, insulin resistance, dyslipidemia, inflammation, hypercoagulability, hypertension, and atherosclerosis. All these factors contribute to endothelial dysfunction and related complications [3].

Type 2 DM (T2DM) is the most common form of diabetes characterized by defects in insulin signaling [4]. Without proper treatment, T2DM can cause severe secondary complications including

Abbreviations: AD, Alzheimer's disease; ADT, AutoDock Tools; CIDs, compound identifiers; DM, diabetes mellitus; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; GAs, genetic algorithms; IR, insulin receptor; IRS, insulin receptor substrate; NCBI, National Center for Biotechnology Information; *p*NPP, *p*-nitrophenyl phosphate; PTP, protein tyrosine phosphatase; PTP1B, protein tyrosine phosphatases 1B; T2DM, type 2 diabetes mellitus

* Corresponding author. Department of Food and Life Science, Pukyong National University, Busan 48513, Republic of Korea.
** Corresponding author. Department of Food Science and Human Nutrition, Chonbuk National University, Jeonju 54896, Republic of Korea.
E-mail addresses: jungha@jbnu.ac.kr (H.A. Jung), choijs@pknu.ac.kr (J.S. Choi).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.cbi.2017.10.013
Received 27 March 2017; Received in revised form 7 October 2017; Accepted 11 October 2017
Available online 12 October 2017
0009-2797/ © 2017 Elsevier B.V. All rights reserved.

atherosclerosis, renal dysfunction, cardiac abnormalities, and ocular disorders [5]. Thus, prevention and treatment options for DM are necessary globally. Insulin resistance is the key pathological characteristic of metabolic syndrome, and shows an unnatural insulin signaling pathway, including the β-subunit of insulin receptor (IR)-β, insulin receptor substrate (IRS) 1/2, and other downstream signal molecules involved in the malfunction of reversible phosphorylation of protein tyrosine and decreased insulin sensitivity of peripheral tissue [6]. Protein tyrosine phosphatase 1B (PTP1B) is a major negative regulator of the insulin signaling containing IR and IRS by dephosphorylation of tyrosine-phosphorylated proteins. Insulin resistance is caused by disequilibrium in IR autophosphorylation and tyrosine kinase activity leading to the overexpression of PTP1B. Thus, PTP1B is a strong potential drug target for insulin sensitivity and insulin resistance.

The dried root of *Salvia miltiorrhiza* BUNGE (Labiatae), called 'Danshen' in China, is very popular traditional Chinese medicine. This plant has been used for the treatment of various diseases such as menstrual disorders, menorrhagia, insomnia, blood circulation diseases, angina pectoris, hyperlipidemia, and inflammation, and is particularly valuable in the treatment of coronary heart diseases [7]. The major constituents of *S. miltiorrhiza* root are abietane-type diterpene pigments (also called tanshinones), which have *ortho*- or *para*-naphthoquinone chromophores [8]. Tanshinones are unique components found in the *Salvia* genus and many exhibit diverse biological activities [9]. Tanshinones, the active components of *S. miltiorrhiza*, have been isolated and used as parenteral solutions in China. Emerging experimental studies and clinical trials have demonstrated that tanshinone IIA prevents atherogenesis as well as cardiac injury and hypertrophy. In atherosclerosis, tanshinone IIA acts by inhibiting LDL oxidation, monocyte adhesion to the endothelium, smooth muscle cell migration and proliferation, macrophage cholesterol accumulation, proinflammatory cytokine expression and platelet aggregation [10]. *In vivo* experiments in rabbits showed that an aqueous extract of *S. miltiorrhiza* induces vasodilation in the coronary, mesenteric, femoral, and renal arteries [11]. A study evaluating the role of PTP1B in high-fat diet-induced cardiac contractile anomalies revealed that PTP1B knockout offers cardioprotection against fatty acid-induced cardiomyocyte contractile anomalies [12]. Similarly, high-fat diet intake compromised cardiomyocyte contractile function as evidenced by decreased peak shortening, maximal velocity of shortening/relengthening, intracellular Ca^{2+} release and prolonged duration of relengthening and intracellular Ca^{2+} decay, the effects of which were alleviated by PTP1B knockout [12]. Blood stasis, a significant pathological product of blood circulation disorders, is often seen in patients with microvascular complications from a high glucose environment [13]. Diabetic microangiopathy, a specific damage to vascular endothelial cells seems to be related to their incapacity to down-regulate uptake of glucose in the case of extracellular hyperglycemia. Similarly, loss of insulin signaling in the endothelium leads to vascular dysfunction and atherosclerosis. It is clear that the link between insulin resistance/hyperinsulinemia and cardiovascular disease is related to dysglycemia, dyslipidemia, hypertension, endothelial dysfunctions and inflammation. Association of diabetes with cardiovascular diseases has been demonstrated genetically [14], biochemically [15], and metabolically [16]. Furthermore, high levels of insulin in the body due to early stages of T2DM competitively inhibit the breakdown of Aβ, resulting in Alzheimer's disease (AD) [17]. Hence, taking in account the biological activity of tanshinones in cardiovascular complications and the association among diabetes, cardiovascular complications and AD, we herein characterized the anti-diabetic potential of tanshinone derivatives.

Thus, in the present study, potential anti-diabetic compounds from *S. miltiorrhiza* root were characterized through PTP1B inhibition, as a promising route for the prevention and treatment of diabetes and its complications.

2. Materials and methods

2.1. Chemicals and reagents

p-Nitrophenyl phosphate (*p*NPP) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). PTP1B (human recombinant) was purchased from Biomols International LP (Plymouth Meeting, PA, USA) and dithiothreitol (DTT) was purchased from Bio-Rad Laboratories (Hercules, CA, USA). All other chemicals and solvents were purchased from E. Merck, Fluka, and Sigma-Aldrich, unless otherwise stated.

2.2. Isolation of compounds

The dried and sliced roots of *S. miltiorrhiza* (10.0 kg) were extracted with 70% ethanol (EtOH, 15 L × 3) at 60 °C under reflux conditions for 3 h. The extract was concentrated *in vacuo* to yield a residue (1.0 kg), which was suspended in H_2O and successively partitioned with chloroform ($CHCl_3$), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). $CHCl_3$ extract (74.3 g) was subjected to silica gel vacuum liquid chromatography with a gradient mixture of hexane:methylene chloride (MC) and MC:EtOAc of increasing polarity as eluents to obtain six fractions (A–F). Fraction A (3.1 g) was subjected to silica gel column chromatography (CC) and eluted with a gradient mixture of hexane:EtOAc (1:0 to 0:1) to yield compound **3** (196.0 mg). Fraction B (18.2 g) was subjected to silica gel CC and eluted with a stepwise gradient of hexane:MC to yield eight subfractions (B.1–B.8). Fraction B.3 (10.3 g) was further separated using silica gel CC with gradient elution of hexane:acetone (1:0 to 0:1) and five subfractions (B.3.1–B.3.5) were obtained. Fraction B.3.2 (746.1 mg) was subjected again to silica gel CC with a gradient mixture of hexane:EtOAc (1:0 to 0:1) as eluent, followed by RP-HPLC with a gradient mobile phase of acetonitrile:H_2O (50–90% for 60 min → 90–100% for 30 min, 6 mL/min) to yield compound **1** (4.0 mg, t_R = 68.0 min). Fraction B.3.4 (1.1 g) was separated using C18 CC, eluted with MeOH:H_2O (6:4 to 1:0) and further purified using RP-HPLC with MeOH:H_2O (75–85%, 6 mL/min for 60 min) to obtain compound **2** (22.5 mg, t_R = 25.5 min). Compound **5** (544.4 mg) was recrystallized from fraction B.3.5 (6.7 g); the portion of B.3.5 remaining in solution was subjected to C18 CC with gradient elution of MeOH:H_2O (1:1 to 1:0) and then purified via RP-HPLC with an isocratic mixture of MeOH:H_2O (72%, 6 mL/min for 60 min) to obtain compound **12** (11.0 mg, t_R = 42.0 min). Fraction B.4 (3.0 g) was chromatographed on silica gel CC with a hexane:acetone gradient mixture (1:0 to 0:1) and recrystallized in MC:MeOH to yield compound **11** (195.0 mg). Fraction B.6 (167.9 mg) was subjected to C18 CC, eluted with MeOH:H_2O (1:1 to 1:0) to yield compounds **8** (10.3 mg) and **9** (18.0 mg) as crystals. Fraction C (9.6 g) was subjected to silica gel CC to collect compounds **4** (450.0 mg) and **10** (300.5 mg). Fraction D (3.9 g) was subjected to silica gel CC with gradient elution of hexane:EtOAc (1:0 to 0:1) and C18 CC with MeOH:H_2O (6:4 to 1:0) to obtain compounds **6** (8.8 mg) and **7** (7.6 mg). Isolated compounds, when compared with the published spectral data, were identified as deoxyneocryptotanshinone (**1**) [18], grandifolia F (**2**) [19], ferruginol (**3**), cryptotanshinone (**4**), tanshinone IIA (**5**), methyl tanshinonate (**9**), 15,16-dihydrotanshinone I (**10**), tanshinone I (**11**) [20], tanshinol B (**6**) [8], tanshinone IIB (**7**) [21], tanshinonal (**8**) [22], and dehydrodanshenol A (**12**) [23]. The purity of these compounds was estimated to be at least 98% based on NMR spectra. The structures of the isolated compounds are shown in Fig. 1.

2.3. PTP1B inhibitory assay

The PTP1B (human recombinant) inhibitory activity of 70% ethanolic extract of *S. miltiorrhiza* root, solvent soluble fractions and their constituents were evaluated using *p*NPP as the substrate [24]. In each well in a 96-well plate (final volume 100 μL), PTP1B enzyme diluted

Download English Version:

https://daneshyari.com/en/article/5559246

Download Persian Version:

https://daneshyari.com/article/5559246

Daneshyari.com