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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research https://doi.org/10.1016/j.apjtm.2017.10.018

Comparative molecular docking studies of lupeol and lupenone isolated from *Pueraria lobata* that inhibits BACE1: Probable remedies for Alzheimer's disease

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ARTICLE INFO

Article history:
Received 21 Aug 2017
Received in revised form 23 Sep 2017
Accepted 25 Oct 2017
Available online xxx

Keywords:
Pueraria lobata
BACE1
Lupeol
Lupenone
Molecular docking
Kinetics

ABSTRACT

Objective: To discover lead lupane triterpenoid's potential isolated from *Pueraria lobata* roots against β-site amyloid precursor protein cleaving enzyme 1 (BACE1), which serve as a rate limiting step in amyloid beta (Aβ) production altering the course of Alzheimer's disease. In addition, enzyme kinetics study and molecular docking were conducted to establish the inhibition type and structure activity relationship.

Methods: A systematic study of 70% ethanolic *P. lobata* root extract was employed to identify its BACE1 inhibitory potential. Further, BACE1 inhibitory potential of two lupane terpenoids, yielded from ethanolic extract, was assessed. In order to determine their inhibition mode, Lineweaver–Burk plots and Michaelis–Menten model for BACE1 was performed. AutoDock 4.2 program in addition determined the molecular interaction of BACE1 with isolated terpenoids.

Results: Considering the inhibitory potential of 70% ethanolic extract *of P. lobata* against BACE1 (IC_{50} = 80.35 μg/mL), lupeol and lupenone were subsequently isolated and exhibited notable or moderate BACE1 inhibitory activity with IC_{50} values of 5.12 and 62.98 μmol/L, respectively, as compared to the positive control quercetin (IC_{50} = 21.28 μmol/L). The enzyme kinetics study enabled us to identify both compounds as competitive inhibitors, where lupeol displayed a very potent inhibition against BACE1 with low inhibition constant (K_i) value of 1.43 μmol/L, signifying greater binding affinity. In order to understand the binding mechanism and structure–activity relationship of two triterpene-based BACE1 inhibitors, we employed computer aided docking studies which evidently revealed that hydroxyl group of lupeol formed two hydrogen bonds with the ASP32 (catalytic aspartic residue) and SER35 residues of BACE1 with the binding energy of (−8.2 kcal/mol), while the ketone group of lupenone did not form any hydrogen bonds with BACE1 giving evidence for less binding affinity. These results in turn have predicted the dependence of the inhibitory activity in the presence of hydroxyl group which has provided a new basis for BACE1 blockade.

Conclusions: Our results have successfully explored the molecular mechanism of lupane triterpenoids via BACE1 inhibition, suggesting that lupeol in particular could be utilized as a useful therapeutic and preventive agent to mitigate Alzheimer's disease.

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Peer review under responsibility of Hainan Medical University.
Foundation project: Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2012R1A6A1028677).
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Please cite this article in press as: Koirala P, et al., Comparative molecular docking studies of lupeol and lupenone isolated from *Pueraria lobata* that inhibits BACE1: Probable remedies for Alzheimer's disease, Asian Pacific Journal of Tropical Medicine (2017), https://doi.org/10.1016/j.apjtm.2017.10.018

1. Introduction

Generally, the accumulation of amyloid beta (Aβ) in fibrillar plagues and soluble oligomers in the higher regions of the brain defines Alzheimer's disease (AD). AD is postulated to be characterized by intracellular neurofibrillary tangles, neuroinflammation, and neuronal dysfunction leading to death. Cumulatively, Aβ is considered the hallmark of AD responsible for triggering a complex pathological cascade leading to neurodegeneration [1]. Further, β-site amyloid precursor protein cleaving enzyme 1 (BACE1) controls the rate limiting step in the production of Aβ responsible for the pathogenesis of AD, which has sought the researchers to target BACE1 for the mitigation of AD [2]. Protein levels of BACE1 are significantly higher in patients having AD, which explains the high importance being given to BACE1 inhibition [3].

Recently, natural products-derived lead compounds for the treatment of AD have increased, as they are free of any potential life threatening side effects. Despite the availability of U.S. Food and Drug Administration approved drugs, like donepezil, tacrine, rivastigmine, and galantamine, for the symptomatic treatment of AD; observable toxic issues such as hepatotoxicity, vomiting, diarrhea, and nausea have forced these drugs out of the pharmaceutical market [4]. *Pueraria lobata* (Wild.) Ohwi (*P. lobata*) also known as kudzu, is a perennial vine native to Asia, primarily subtropical and temperate regions of China, Japan, and Korea, whose roots can account for up to 40% of the total plant biomass [5,6]. The starch extracted from its roots is used as herbal medicines, and foods, including naengmyeon. In China, the root is used in herbal remedies and mostly in teas. Recent research has focused on a kudzu root-derived medicinal product for alcohol-related problems [7]. *P. lobata* roots are known to exhibit antioxidant [8], anti-inflammatory [9], hepatoprotective [10], anti-diabetic [11], anti-dipsotropic [12], anti-atherogenic activities [13], hypolipidemic [14], and anti-obesity [15]. Kudzu also has compounds that display estrogenic activity [16] and is used for cardiovascular treatment [17]. A group of terpenes with particular importance are triterpenes that have been identified and classified according to their structures and chemical properties, and can be found in the form of aglycones or as free acids. Emphasizing from a biological perspective, the most important triterpenes are the pentacyclic oleanane, ursane, and lupane that are abundant in higher plants [18]. Lupeol and lupenone from *P. lobata* are pentacyclic triterpenes of 30-carbon skeleton, comprising 4 six membered rings and 1 five membered ring [19,11]. Substantial research over the last three decades has uncovered several important pharmacological activities of lupeol, establishing it as a magical drug. Lupenone and lupeol have been accounted with diverse bioactivities, including anti-inflammatory [20], antioxidant [21], antitumor [22], immunomodulatory [23], antileishmanial [24], and antibacterial [25]. Despite several efforts have been attempted to clarify the pharmacological activities of lupeol and lupenone, the stereochemistry on BACE1 have not been characterized yet. Thus, the knowledge on structure activity relationship (SAR) of terpenoids interacting with BACE1 is vital to understand the enzyme target. Our study aimed to provide the comparative inhibition effect of lupeol and lupenone against BACE1 highlighting its importance. Only limited researches regarding the stereochemistry of lupeol have been attempted. Binding mode on protein tyrosine phosphatase 1B [26], urinary tract infection [27], and cancer [28] have been demonstrated by various literature. Therefore, the molecular basis for binding to the active site of BACE1 is elucidated via computer aided molecular binding analysis, which is believed to be the first of its kind regarding BACE1.

2. Materials and methods

2.1. Chemicals and reagents

A BACE1 FRET assay kit was purchased from PanVera Co. (Madison, WI, USA). All the required reagent-graded chemicals used in this study were bought from commercial sources.

2.2. Plant materials

P. lobata were gathered from Gangwon-do Province, Korea, in March 2015, and validated by Prof. Jae Sue Choi. A voucher specimen (20150320) was deposited in the Prof. Choi's lab.

2.3. Extraction and fractionation

The dried root of *P. lobata* (1 kg) after extraction with distilled water gave 175 g of extract. The dried root of *P. lobata* (3 kg) was again separately extracted with 70% ethanol (EtOH) under reflux, yielding 0.9 kg of extract. The 70% EtOH extract was then fractionated with different soluble solvents to yield *n*-hexane (27.5 g), dichloromethane (4.3 g), ethyl acetate (22.2 g), and *n*-butanol (391 g) fractions, as well as water residue (455 g).

2.4. Isolation of compounds

Lupenone (234 mg) and lupeol (550 mg) were isolated from the *n*-hexane fraction (27.5 g), that were elucidated via some spectroscopic methods, including proton and carbon-NMR, as well as through the published spectral data [11,29].

2.5. In vitro BACE1 enzyme assay

Assays were performed using the commercial protocol, BACE1 FRET assay kit (PanVera Co.) method with slight modification. Quercetin was used as a standard.

2.6. Type of inhibition of lupeol and lupenone towards BACE1 using enzyme kinetics

To determine the kinetic mechanisms of lupeol and lupenone towards BACE1, we produced Lineweaver–Burk plot and Michaelis–Menten model by varying concentrations of substrate (0–750 nmol/L) and inhibitors (0–14 μmol/L for lupeol and 0–120 μmol/L for lupenone) [30]. Kinetic parameters, including inhibition constants (K_i), maximum reaction velocity (V_{max}), and Michaelis Menten constant (K_m) values were calculated via Sigmaplot 12.0 (Systat Software Inc., San Jose, CA) [31,32].

2.7. Molecular docking simulations

AutoDock 4.2 software was employed to assess the structure of the enzyme-inhibitor complex. In our study, lupeol and lupenone were tested for BACE1 inhibition. AutoDock 4.2 predicts binding free energies of enzyme-inhibitor complexes and the

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