Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Auriculasin from Flemingia philippinensis roots shows good therapeutic indexes on hyperactive behavior in zebrafish

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ARTICLE INFO

Article history:
Received 29 June 2018
Accepted 7 July 2018
Available online xxx

Keywords:
Isoflavones
ADHD
Hyperactive behavior
Zebrafish
Circadian clock
per1b

ABSTRACT

Previously, *period1b*−/− zebrafish mutants were used to establish an attention deficit hyperactivity disorder (ADHD) model, in which hyperactive behavior was found to be a typical characteristic of ADHD due to down-regulated dopamine levels. Here, we used five prenylated isoflavones from *Flemingia philippinensis* roots to study their therapeutic effects on hyperactivity behavior in *period1b*−/− zebrafish. Results of locomotor activity assay showed that auriculasin, one of the prenylated isoflavones, significantly reduced the hyperactivity behavior in *period1b*−/− zebrafish. Hormone measurement results showed that auriculasin increased melatonin and dopamine content. Results of quantitative real-time polymerase chain reaction showed that auriculasin down-regulated the expression of *mao* but up-regulated the expression of *th* and *per1b*. Thus, auriculasin demonstrated a potential biological effect on dopamine activity to inhibit hyperactivity behavior in the ADHD zebrafish model by regulating circadian clock gene per1b.

1. Introduction

Isoflavones are diphenolic compounds present in many plants such as soybeans, Mexico small white beans, alfalfa, and green beans [1]. Soybeans are the most common dietary source of isoflavones [2]. Studies on human nutrition have shown that soybean isoflavones play an important role in preventing a number of chronic diseases [3,4]. Soy protein and isoflavones may decrease the risk factors associated with cardiovascular disease and protect against spinal bone loss [5,6]. Epidemiological studies have shown the association of soybean isoflavones, as dietary components, with lower risk of prostate and colorectal cancer in Asian than in Western populations [7,8].

Flemingia philippinensis belongs to the family of legumes and has been cultivated as a traditional Chinese medicine in tropical parts of China. *F. philippinensis* roots contain various isoflavones, which have anti-inflammatory effects and help in nerve injury repairs [9]. Moreover, isoflavones can regulate cell apoptosis [10,11], have antioxidant properties, and modulate immune and inflammatory responses [12,13]. Moreover, studies showed that isoflavones are phytoestrogens [14] that can bind to and induce transcriptional activity of estrogen receptors, which affect dopamine-dependent cognitive processes in females [15,16].

Attention deficit hyperactivity disorder (ADHD) is a highly heritable psychiatric condition with a worldwide prevalence in childhood and adolescence [17,18]. ADHD is characterized by a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with social, academic, or occupational functioning [19]. These symptoms are chronic; persist in adulthood in approximately half of the affected individuals; and are associated with impaired family and peer relationships, increased risk of drug abuse and criminality, and significantly increased mortality rates [20,21].

In a previous study, *period1b*−/− zebrafish mutants clearly displayed altered circadian rhythms, defective learning and memory, hyperactivity, and impulsivity-like symptoms [22]. These *period1b*−/− zebrafish mutants were then used to establish an ADHD model, and the hyperactive behavior was found to be a typical characteristic of ADHD due to down-regulated dopamine

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https://doi.org/10.1016/j.bbrc.2018.07.033
0006-291X/

levels [22].

Dopamine agonists are a pharmacological class of drugs that effectively act on the nervous system for abnormal behavior, including Parkinson's disease and restless legs syndrome [23]. The dopamine agonists include bromocriptine, pergolide, pir-ibedil, lisuride, cabergoline, pramipexole, ropinirole, Ritalin, and apomorphine [24,25]. These dopamine agonists are currently regulated mainly due to their adverse effects including valvular heart diseases, sleep attacks, and impulse control disorders [26]. Here we used five prenylated isoflavones [9], including flemiphilippinin A, flemiphilippinin E, 6,8-diprenylorobol, flemingsin, and auriculasin from *F. philippinensis* roots to study their effects on dopamine activity and hyperactive behavior in *period1b*−/− zebrafish mutants. We found that auriculasin increased melatonin and dopamine content in *period1b*−/− zebrafish. Moreover, results of quantitative real-time polymerase chain reaction (qRT-PCR) showed that auriculasin down-regulated the expression of *ma* but up-regulated the expression of *th* and *per1b*. Therefore, auriculasin demonstrated a potential biological effect on dopamine activity to inhibit hyperactivity behavior in *period1b*−/− zebrafish by regulating circadian clock gene per1b.

2. Materials and methods

2.1. Drugs

Five prenylated isoflavones were extracted from *F. philippinensis* roots using five different solvent extraction systems: chloroform, 50% ethanol, ethanol, methanol, and distilled water as previously described [9]. Prenylated isoflavones 1 (PI1) is Flemiphilippinin A; prenylated isoflavones 2 (PI2) is Flemiphilippinin E; prenylated isoflavones 3 (PI3) is 6,8-Diprenylorobol; prenylated isoflavones 4 (PI4) is Flemingsin; prenylated isoflavones 5 (PI5) is Auriculasin [9] (Fig. 1A). Ritalin, the trade names of methylphenidate (MPH), is bought from Sigma-Aldrich (Fig. 1A).

2.2. Zebrafish maintenance

Wild-type zebrafish (*Danio rerio*) AB strain and *period1b*−/− mutant fish were maintained under 14 h light/10 h dark cycle at 28 °C in the Soochow University Zebrafish Facility according to standard protocols. We screened the *period1b*−/− zebrafish mutants, in which the retroviral sequence was inserted into the first intron, as previously described [22,27]. All animal experiments

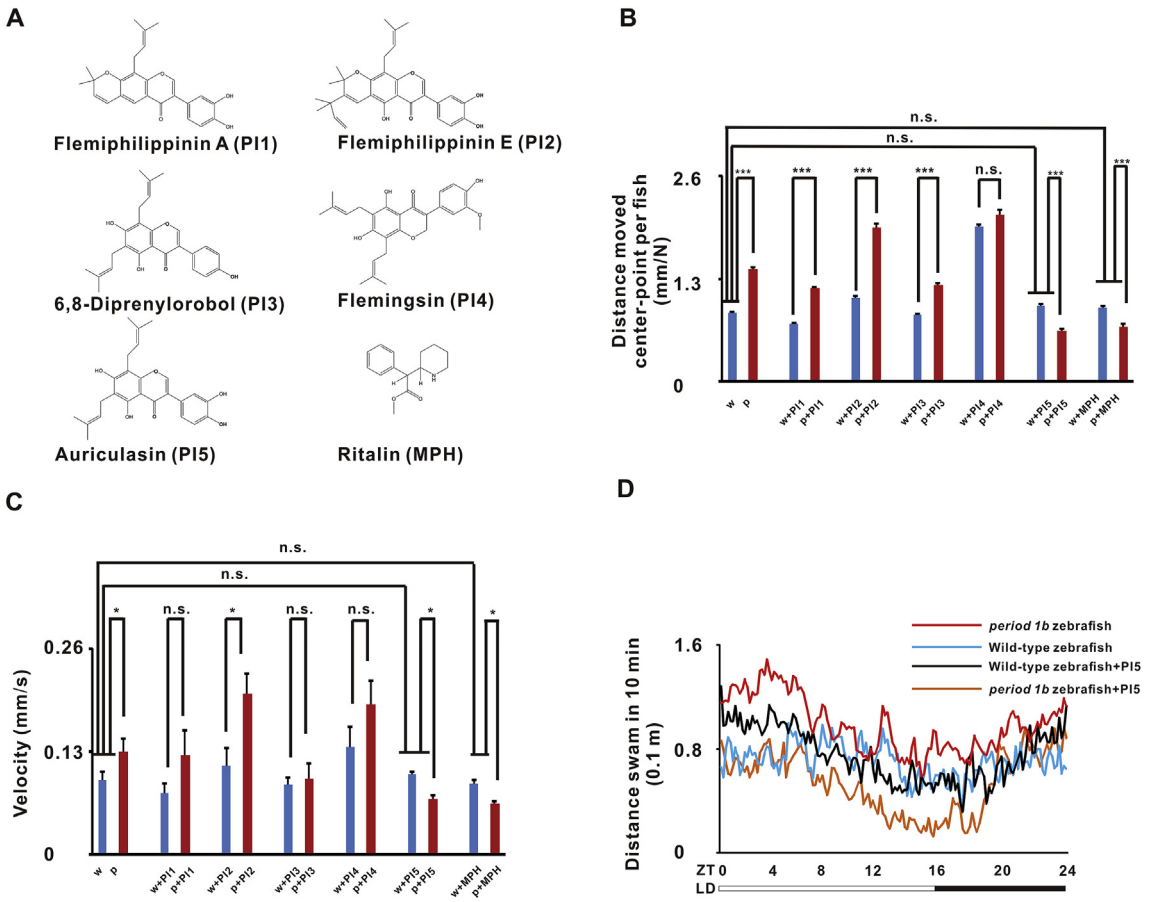

Fig. 1. Effects of five prenylated isoflavones on zebrafish locomotor activities. (A) Chemical structures of five prenylated isoflavones from the root of *F. philippinensis* and Ritalin. (B) Distance from center point per movement. (C) Average velocity. (D) Wild-type and *period1b*−/− adult mutants displayed significantly down-regulated locomotor activities after auriculasin treatment. "w" represents wild-type larvae; "w + PI1" represents wild-type larvae treated with 20 nmol flemiphilippinin A; "w + PI2" represents wild-type larvae treated with 20 nmol flemiphilippinin E; "w + PI3" represents wild-type larvae treated with 20 nmol 6,8-diprenylorobo; "w + PI4" represents wild-type larvae treated with 20 nmol flemingsin; "w + PI5" represents wild-type larvae treated with 20 nmol auriculasin; "w + MPH" represents wild-type larvae treated with 20 nmol Ritalin; "p" represents *period1b*−/− larvae; "p + PI1" represents *period1b*−/− larvae treated with 20 nmol flemiphilippinin A; "p + PI2" represents *period1b*−/− larvae treated with 20 nmol flemiphilippinin E; "p + PI3" represents *period1b*−/− larvae treated with 20 nmol 6,8-diprenylorobo; "p + PI4" represents *period1b*−/− larvae treated with 20 nmol flemingsin; "p + PI5" represents *period1b*−/− larvae treated with 20 nmol auriculasin; "p + MPH" represents *period1b*−/− larvae treated with 20 nmol Ritalin. One-way ANOVA was conducted. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Data represent the mean and SD of five independent experiments.

Please cite this article in press as: T. Wang, et al., Auriculasin from Flemingia philippinensis roots shows good therapeutic indexes on hyperactive behavior in zebrafish, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.033