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Metabolism

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Basic Science

Albiflorin ameliorates obesity by inducing thermogenic genes via AMPK and PI3K/AKT in vivo and in vitro

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ARTICLE INFO

Article history:
Received 22 December 2016
Accepted 26 May 2017

Keywords:
Albiflorin
hAMSCs
High-fat diet-induced obesity
Brown adipose tissue
Thermogenic gene

ABSTRACT

Objective. Brown adipose tissue (BAT) activation has been identified as a possible target to treat obesity and to protect against metabolic diseases by increasing energy consumption. We explored whether albiflorin (AF), a natural compound, could contribute to lowering the high risk of obesity with BAT and primary brown preadipocytes in vivo and in vitro.

Materials/Methods. Human adipose tissue-derived mesenchymal stem cells (hAMSCs) were cultured with adipogenic differentiation media with or without AF. Male C57BL/6J mice (n = 5 per group) were fed a high-fat diet (HFD) for six weeks with or without AF. Brown preadipocytes from the interscapular BAT of mice were cultured with or without AF.

Results. In white adipogenic differentiation of hAMSCs, AF treatment significantly reduced the formation of lipid droplets and the expression of adipogenesis-related genes. In HFD-induced obese C57BL/6J mice, AF treatment significantly reduced body weight gain as well as the weights of the white adipose tissue, liver and spleen. Furthermore, AF induced the expression of genes involved in thermogenic function in BAT. In primary brown adipocytes, AF effectively stimulated the expressions of thermogenic genes and markedly up-regulated the AMP-activated protein kinase (AMPK) signaling pathway. Pretreatment with phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002 nullified the induction of the thermogenic genes by AF in

Abbreviations: ACC, Acetyl coenzyme A carboxylase; AF, Albiflorin; AKT, Protein kinase B; ALT, Alanine transaminase; AMPK, AMP-activated protein kinase; AP2, Fatty acid-binding protein 4; AST, Aspartate transaminase; BAT, Brown adipose tissue; CD137, Cluster of differentiation-137; CEBPα, CCAAT/enhancer binding protein α; CIDEA, Cell-death-inducing DFFA-like effector-a; GLUT4, Glucose transporter 4; hAMSCs, Human adipose tissue- derived mesenchymal stem cells; HFD, High-fat diet; H&E, Hematoxylin and eosin; LKB1, Liver kinase B 1; MTOR, Mammalian target of rapamycin; NRF1, Nuclear respiratory factor 1; PGC1α, Peroxisome proliferator-activated receptor γ coactivator 1α; PI3K, Phosphatidylinositol 3-kinase; PPARγ, Peroxisome proliferator-activated receptor γ; SIRT3, Sirtuin 3; TMEM26, Transmembrane 26; UCP1, Uncoupling protein 1; WAT, White adipose tissue.

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http://dx.doi.org/10.1016/j.metabol.2017.05.009

primary brown adipocytes. Moreover, AF activated beige cell marker genes induced by the pharmacological activation of peroxisome proliferator-activated receptor γ in hAMSCs.

Conclusion. This study shows that AF prevents the development of obesity in hAMSCs and mice fed an HFD and that it is also capable of stimulating the differentiation of brown adipocytes through the modulation of thermogenic genes by AMPK and PI3K/AKT.

1. Introduction

Obesity is a major risk factor for type 2 diabetes mellitus (T2DM) including other chronic metabolic disorders and has become a health problem across the world [1,2]. In mammals, there are two functionally and morphologically distinct types of adipose tissue, white and brown, which have opposite functions with regard to their energy balance. White adipocytes store excess energy as triglycerides in lipid droplets, whereas brown adipocytes release energy in the form of heat through thermogenesis [3]. The thermogenesis capacity of brown adipocytes results from the expression of a brown fat-specific mitochondrial inner membrane protein, uncoupling protein 1 (UCP1), leading to the dissipation of chemical energy as heat [4]. Brown adipocytes are very prominent in rodents and human infants [5]. The amount of brown adipose tissue (BAT) in adult humans has been found to be highly inversely correlated with their degree of obesity, indicating that the activation of brown adipocytes may have an important role in the prevention of obesity [6,7]. Current evidence suggests that there are at least two types of brown adipocytes, "classical" brown adipocytes that reside in the interscapular BAT depot [8] and "beige" or "brite" cells which are sporadically found in the white adipose of adult animals that have been chronically exposed to cold or peroxisome proliferator-activated receptor γ (PPARγ) ligands. These "inducible" brown adipocytes possess the morphological and biochemical characteristics of classical brown adipocytes [9]. Mesenchymal stem cells from human adipose tissue which exhibit a self-renewal ability are able to convert into functional brown-like adipocytes under certain conditions [10]. Therefore, targeting BAT in adults may offer an effective treatment for obesity and related metabolic disorders.

AMP-activated protein kinase (AMPK) is one of the key regulators in energy homeostasis, as it regulates glucose and lipid metabolism to modulate energy levels. The activation of AMPK induces the translocation of glucose transporter 4 (GLUT4) to the plasma membrane [11]; therefore, AMPK signaling pathways are potential molecular targets for the treatment of type 2 diabetes and obesity [12,13]. It has been reported that AMPK is a stress-responsive inhibitor of mammalian target of rapamycin (mTOR) activation [14]. Persistent mTOR activation is associated with diverse pathologies such as cancer, diminished cardiac performance, and obesity-associated metabolic diseases [15]. Accordingly, chronic pharmacological inhibition of the mTOR pathway is also associated with a reduction in obesity [16]. Phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathways have crucial roles in adipocyte differentiation. For instance, addition of PI3K inhibitors completely blocks the differentiation process [17]. Additionally, this signal was reported to be involved in the process of brown adipogenesis as well [18].

Albiflorin (AF), a monoterpene glycoside, is one of the major compounds of *Paeonia lactiflora* Pallas. In some Asian countries including China and Korea, *Paeonia lactiflora* Pallas has been used to treat inflammation, thrombosis, cancer, atherosclerosis, and depression [19–23]. Unlike paeoniflorin, another monoterpene glycoside extract of *Paeonia lactiflora* Pallas, the biological activity of AF has only been investigated in a few studies. It has recently been proposed that AF is able to attenuate the epididymal adiposity and glucose intolerance induced by a chronic high-fat diet (HFD) [24].

In this study, we investigated the anti-obesity effect of AF with human adipose tissue-derived mesenchymal stem cell (hAMSC) and diet-induced obese mice as well as the thermogenic activity of AF with BAT and then confirmed the mechanisms by which AF mediates the thermogenic activity in brown adipocytes.

2. Materials and Methods

2.1. hAMSC Cultures

hAMSCs used in this study were purchased from CEFO Bio (Seoul, Korea). Cells were grown in hAMSC growth media (CEFO Bio) at 37 °C in a 5% CO_2 humidified atmosphere. Adipogenic differentiation of hAMSCs was done as previously described [25]. For white/brown adipogenic differentiation, hAMSCs were cultured in growth media up to confluence. White adipogenesis was induced by culturing hAMSCs for 14 days in adipogenic induction medium [10% FBS, 1 μmol/l insulin, 0.5 mmol/l 3-isobutyl-1-methylxanthine (IBMX), 1 μmol/l dexamethasone (DEX), and 100 μmol/l indomethacin in Dulbecco's Modified Eagle's Medium (DMEM)] and adipogenic maintenance medium (10% FBS and 1 μmol/l insulin in DMEM). The medium was changed every 3 days. On day 6, the adipogenic induction medium was replaced with adipogenic maintenance medium. For brown-like adipogenic differentiation, the hAMSCs were then maintained in DMEM media supplemented with 10 μg/ml of transferrin, 0.85 μmol/l insulin, 0.2 mol/l T3, 1 μmol/l DEX, and 0.5 mmol/l IBMX. Three days later, the medium was changed (DEX and IBMX omitted) and 1 μmol/l troglitazone was added for the indicated periods. Media were then changed every other day, and the cells were used at the indicated days.

2.2. Animal Studies

All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Review Board of Kyung Hee University (confirmation number: KHUASP (SE)-13-012). Male C57BL/6J mice (4-week-old) were purchased from Daehan