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Inhibitory effect and transcriptional impact of berberine and evodiamine on human white preadipocyte differentiation

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ABSTRACT

It has been reported that the botanical alkaloids, berberine and evodiamine inhibit mouse preadipocyte 3T3-L1 differentiation. The aim of this study was to investigate the effect and transcriptional impact of berberine and evodiamine individually and in combination on human white preadipocyte (HWP) differentiation. We have shown that treatment with 8 μM berberine or 4 μM evodiamine resulted in a major inhibition of HWP differentiation accompanied by up-regulation of both GATA binding protein 2 and 3 (GATA-2 and GATA-3) mRNA and protein expression, suggesting that both compounds may have excellent potential as agents to prevent obesity.

1. Introduction

There is irrefutable evidence that obesity is a threat to world health. The worldwide obesity epidemic has been strongly associated with the major risks of developing diseases such as type 2 diabetes, hyperlipidemia, hypercholesterolemia, hypertension and cancer [1–3] and has led to a vastly increased number of preventable deaths [4]. Thus, the prevention and treatment of obesity is critical in maintaining worldwide health. In obese individuals, the differentiation process of preadipocyte into mature adipocyte is the critical check point in the development of obesity [5]. Although adipocyte differentiation is a complex process controlled by multiple regulators, the major players are peroxisome proliferator activated receptor γ (PPARγ) [6] and CCAAT/enhancer binding protein α (C/EBPα) [7]. More recently the transcription factors GATA binding protein 2 and 3 (GATA-2 and GATA-3) have been shown to be important gate keepers of the differentiation process [8–10].

Berberine (Fig. 1A) is an alkaloid isolated from many medicinal herbs and is a major component of the Chinese medicine, Huang-Lian. Traditionally used extensively as an anti-bacterial drug [11], berberine has been proven to have many other pharmacological effects including antimicrobial [12], antitumor [13], anti-inflammation [14], as well as LDL-lowering effects [15] and also body weight reduction [16]. Several research groups have reported and confirmed that berberine inhibits mouse 3T3-L1 preadipocyte differentiation via mechanisms involving down-regulation of the adipogenic transcription factors C/EBPα and PPARγ [17], inhibiting IkB kinase β(IKKβ) [18], activation of AMP kinase [19,20] and activating glucose transporter 1 (GLUT1) [21]. The previous work of our group has demonstrated that the inhibitory effects of berberine on the differentiation of murine 3T3-L1 cells were accompanied both by increased GATA-2 and GATA-3 mRNA and protein expression [22].

Evodiamine (Fig. 1B) is an alkaloid and one of the major bioactive compounds present in the Chinese medicine, Wu-Chu-Yu. Evodiamine is also known to possess anti-inflammatory

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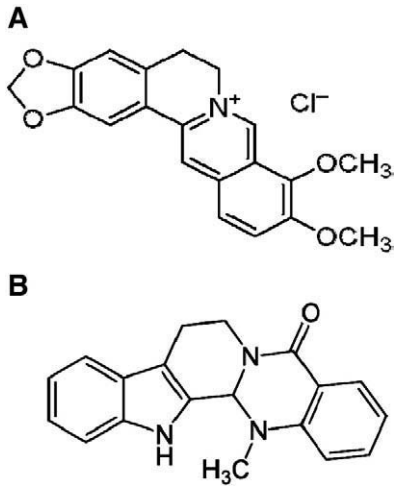

Fig. 1. Chemical structure of berberine chloride (A) and evodiamine (B).

[23], antitumor [24,25] and anti-obesity [26] properties and was recently reported to not only inhibit 3T3-L1 cell differentiation but also decrease weight-gain in diet-induced obesity of Ucptm1 knockout mice [27]. In addition, it was reported that evodiamine functioned as an agonist for rat transient receptor potential vanilloid type-1 (TRPV1) [28] and the activation of TRPV1 prevented adipogenesis in 3T3-L1 preadipocyte and visceral adipose tissue from mice and humans [29], which suggests that evodiamine could inhibit adipogenesis with high possibility during human white preadipocyte differentiation. Huang-Lian and Wu-Chu-Yu have been used in Chinese medicine both individually and in combination to treat a variety of syndromes for thousands of years.

Although berberine and evodiamine have been demonstrated to inhibit the differentiation of murine preadipocyte [30], and the mechanisms in murine models of adipogenesis have been fairly well investigated, human models of adipogenesis are relatively poorly characterized. Investigations have, in the past, been hampered by a lack of suitable human preadipocyte cell lines [31]. However, in recent years a number of advances in the development of human preadipocyte cell lines have facilitated more in-depth differentiation studies resulting in several groups using primary human preadipocyte to investigate the differentiation process [32–34]. Nevertheless, the effects of berberine and evodiamine either individually or in combination on the differentiation process of human preadipocyte have not been reported.

In this report, we present evidence that both berberine and evodiamine individually and in combination inhibit the differentiation of human white preadipocyte (HWP) and increase the GATA-2 and GATA-3 gene and protein expression.

2. Materials and methods

2.1. General

Berberine and evodiamine were purchased from Sigma-Aldrich Co, St. Luis, MO, USA. Optical rotation of evodiamine was measured in chloroform on AUTOPOL IV automatic polarimeter.

2.2. Cell culture

Cell culture was carried out following the protocol provided by Promocell Company, Heidelberg, Germany. Briefly, human white preadipocytes (HWP 32/female/Caucasian) were cultured at 37 °C in a humidified 5% CO_2 atmosphere and grown in a preadipocyte Growth medium (GM) with 100 U/ml penicillin and 100 μg/ml streptomycin until confluence (day 0). Differentiation was induced with preadipocyte Differentiation medium (DM) for 3 days (day 3), where upon the medium was changed to adipocyte Nutrition medium (NM) and cultured for 12 days (day 15) (HWP cell line and media were purchased from Promocell, Heidelberg, Germany). Varying concentrations of berberine, evodiamine, and their combination were added to DM and NM in order to observe their effects.

2.3. MTT assay

To detect the effect of berberine, evodiamine and their combination on the viabilities of HWP during differentiation induction, HWP were plated in 96-well culture plates at a density of 4×10^3 cells/well and cultured in GM until confluent, then cultured in DM and NM supplemented with varying concentrations of berberine and evodiamine, alone and in combination. Medium was removed at different time points and MTT (0.5 mg/ml in NM, 50 μl/well) was added. The plates were incubated at 37 °C for 4 h, followed by the addition of DMSO (150 μl/well), and incubated at 37 °C for 1 h. Optical density (OD) was measured at 570 nm with 650 nm as background.

2.4. Oil-Red-O staining and quantification

Oil-Red-O staining and quantitative Oil-Red-O staining were performed as previously reported [35,36]. Briefly, medium was removed and cells were washed with PBS twice, fixed with 3.7% formalin at room temperature for 30 min, then rinsed with water, added 60% 2-propanol and incubated for 5 min, then moved out 2-propanol and stained cells with Oil-Red-O solution (Oil-Red-O store solution (3 mg/ml in pure 2-propanol): water = 3:2) at room temperature for 10 min. Then the Oil-Red-O solution was removed and cells were washed 3 times with water. Images were obtained using an Olympus IX70 inverted microscope equipped for phase-contrast microscopy (Olympus, Tokyo, Japan). After staining, the cells were washed twice with 70% ethanol to remove excess stain. Stained oil droplets in the cells were dissolved in 2-propanol containing 4% Nonidet-P40 (Fisher Scientific, Pittsburgh, PA, USA) and OD values were measured at an absorbance of 490 nm.

2.5. Quantitative Real-Time RT-PCR

Real-Time reverse transcriptase polymerase chain reaction (RT-PCR) analysis was used to measure mRNA expression of human genes PPARγ, C/EBPα, GATA-2 and GATA-3 under the control of β-actin. Briefly, total RNA was isolated from HWP following treatment at day 15 with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. RNA was quantified using absorption of