Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 16 (2009) 864–873
Phytomedicine
www.elsevier.de/phymed

Berberine increases expression of GATA-2 and GATA-3 during inhibition of adipocyte differentiation

Y. Hu[a], G.E. Davies[a,b,c,*]

[a]*Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, SD 57007, USA*
[b]*Department of Psychiatry, Sanford School of Medicine, University of South Dakota, SD, USA*
[c]*Avera Institute for Human Behavioral Genetics, Avera Behavioral Health Center, Sioux Falls, SD, USA*

Abstract

It is known that a number of transcription factors are key regulators in the complex process of adipocyte differentiation including peroxisome proliferator activated receptor γ (PPARγ) and the CCAAT enhancer binding protein α (C/EBPα). Studies have demonstrated that in pre-adipocyte 3T3-L1 cells constitutive expression of the DNA binding proteins GATA-2 and GATA-3 results in protein/protein interactions with C/EBPα resulting in down regulation of PPARγ and subsequent suppressed adipocyte differentiation with cells trapped at the pre-adipocyte stage. Thus it appears that GATA-2 and GATA-3 are of critical importance in regulating adipocyte differentiation through molecular interactions with PPARγ and C/EBPα. Recent reports suggest that berberine, an isoquinoline derivative alkaloid isolated from many medicinal herbs prevents differentiation of 3T3-L1 cells via a down regulation of PPARγ and C/EBPα expression. The aim of this study was to determine the effect of berberine on GATA-2 and 3 gene and protein expression levels during differentiation of 3T3-L1 cells. MTT (Methylthiazolyldiphenyl-tetrazolium bromide) was used to detect the cytotoxic effects of berberine on the viability of 3T3-L1 cells during proliferation and differentiation. Differentiation of 3T3-L1 cells was monitored by Oil Red O staining and RT-PCR of PPARγ and C/EBPα and the expression of GATA-2 and 3 was determined by RT-PCR and Western Blot. Results show that following treatment with 8 μM berberine the mRNA and protein expression levels of GATA-2 and 3 were elevated and accompanied by inhibited adipocyte differentiation. These results may lead to the use of berberine to target the induction of specific genes such as GATA-2 and GATA-3 which affect adipocyte differentiation.

Keywords: Berberine; 3T3-L1; Adipocyte differentiation; GATA; PPARγ; C/EBPα

Introduction

There is overwhelming evidence that obese individuals are more likely than their lean counterparts to develop cardiovascular disease, Type II diabetes, hypertension, hyperlipidemia, and hypercholesterolemia (Björntorp 1990). The increase in adiposity in these individuals results from an escalation in adipocyte number and an augmentation in size of individual fat cells. Additionally, the disproportionate increase in the visceral adipose deposits in some individuals is linked to development of certain metabolic disorders. The prevention and treatment of obesity has become a critical world health issue. In obese or overweight individuals, the differentiation of pre-adipocyte into mature adipocyte plays a major role

*Corresponding author at: Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, SD 57007, USA. Tel.: +1 605 688 4090; fax: +1 605 688 6232.
E-mail address: gareth.davies@sdstate.edu (G.E. Davies).

0944-7113/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2009.03.002

in the development of obesity (Auwerx et al. 1996). Consequently, understanding the complex pathways and mechanisms regulating adipose formation should provide valuable information in the fight to combat the growing epidemic of obesity in the modern world. Differentiation of pre-adipocyte into adipose tissue requires several transcription factors and at the center of this network are the two principal adipogenic factors, CCAAT/enhance-binding proteins α (C/EBPα) (Lane et al. 1996) and peroxisome proliferator activated receptor γ (PPARγ) (Hamm et al. 1999) which oversee the entire terminal differentiation process. PPARγ, a nuclear hormone receptor, is of particular importance as it functions as a transcriptional regulator in adipose tissue controlling multiple genes involved in lipid and glucose metabolism (Lee et al. 2003). PPARγ is considered the master regulator of adipogenesis. Without it, precursor cells are incapable of expressing any known aspect of the adipocyte phenotype (Rosen et al. 2002). On the other hand, cells deficient in C/EBPα are capable of adipocyte differentiation. However, these C/EBPα-deficient cells are insulin resistant (El-Jack et al. 1999; Wu et al. 1999). Knowledge of this complex network and the importance of PPARγ and C/EBPα arise predominantly from studies performed in established pre-adipocyte cell lines. More recently, data from a variety of knockout mice have confirmed these *in vitro* studies showing that many components of this network are required as regulators of adipocyte development and function.

Berberine is a plant alkaloid with a long history of medicinal use in Chinese and Ayurvedic medicine. Berberine is present in the roots, rhizomes, and stem bark of seven plant families including *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). The chemical structure of berberine used in this study is shown in Fig. 1. Studies have shown that berberine possesses many and varied pharmacological properties and has potential use as an anti-cancer agent, as an antimicrobial and in lowering LDL (Anis et al. 2001; Huang and Williams 1999; Auwerx et al. 1992). Recent reports have shown berberine as a potential inhibitor of pre-adipocyte differentiation (Choi et al. 2006; Huang et al. 2006) and Huang et al. (2006) reported that the possible mechanism of inhibiting pre-adipocyte differentiation is related to down-regulation of the transcription factors C/EBPα and PPARγ.

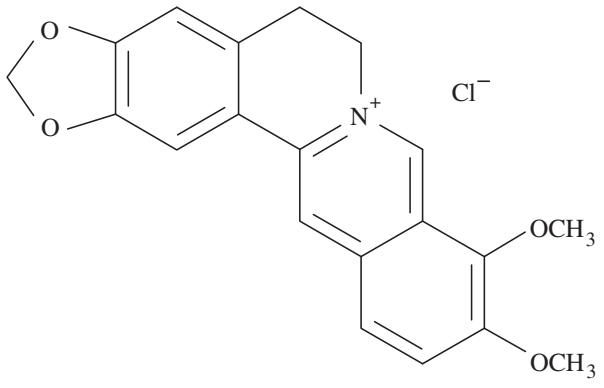

Fig. 1. Chemical structure of berberine chloride.

It has been reported that the transcription factors GATA binding protein 2 and 3 (GATA-2 and GATA-3) are expressed in adipocyte precursors and control the pre-adipocyte to adipocyte transition. Studies show that constitutive expression of both GATA-2 and GATA-3 suppressed adipocyte differentiation (Tong et al. 2000). The mechanism is partially through direct binding to the peroxisome proliferators activated receptor γ (PPARγ) promoter and suppression of its basal activity. In addition, both GATA-2 and GATA-3 form protein complexes with CCAAT/enhancer binding protein α (C/EBPα) (Tong et al. 2005). However, whether the berberine can directly affect the expression of GATA-2 and 3 has not been investigated. In this study, we have examined the effects of berberine on the differentiation of 3T3-L1 cells and demonstrated that berberine increases GATA-2 and GATA-3 mRNA and protein expression accompanied by inhibited adipocyte differentiation.

Materials and methods

Materials

3T3-L1 cells, Dulbecco's Modified Eagle's Medium (DMEM) were purchased from ATCC Global Bioresource Center, Fetal Bovine Serum (FBS) was purchased from Atlanta Biologicals Corp., Berberine chloride, Penicillin/streptomycin, Oil Red O, MTT, Isopropanol, Acrylamide/Bis-Acrylamide, DMSO, DNA size markers were purchased from Sigma Co., Trizol and Superscript One-Step RT-PCR kit were purchased from Invitrogen Life Technologies, Oligonucleotide primers were synthesized by Integrated DNA Technologies Inc., Antibodies were purchased from Santa Cruz Biotechnology Inc., Whatman nitrocellulose membrane was purchased from Thermo Fisher Scientific, ECL detection kit was purchased from GE Healthcare. Nonidet P40 was bought from Fisher Scientific Co..

Cell culture

3T3-L1 cells were cultured at 37 °C in a humidified 5% CO^2 atmosphere and grown in a culture medium (CM) (DMEM supplemented with 10% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin).