Life Sciences 148 (2016) 112–117

Contents lists available at ScienceDirect

Life Sciences

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

Pharmacological inhibition of galectin-1 by lactulose alleviates weight gain in diet-induced obese rats

Rajib Mukherjee, Jong Won Yun *

Department of Biotechnology, Daegu University, Kyungsan, Kyungbuk 712-714, Republic of Korea

ARTICLE INFO

Article history:
Received 13 November 2015
Received in revised form 31 January 2016
Accepted 5 February 2016
Available online 13 February 2016

Keywords:
Adipogenesis
Adipose tissue
Galectin-1
Lactulose
Obesity

ABSTRACT

Aims: Galectin-1 (GAL1) is an important member of the lectin family with a carbohydrate recognition domain and has recently been demonstrated to be involved in adipose metabolism. In the present study, we investigated the effects of targeted inhibition of GAL1 by its binding inhibitor lactulose under high fat diet (HFD)-induced obesity.

Main methods: Effects of targeted inhibition of GAL1 by lactulose on lipid metabolism were investigated *in vitro* and *in vivo*. Changes in lipogenic capacity in lactulose-treated adipocytes were demonstrated by Oil Red O staining, triglyceride quantification and major adipogenic marker expression patterns. After lactulose treatment in Sprague-Dawley rats, various important body weight parameters, food efficiency, plasma metabolic parameters (glucose, ALT, free fatty acid, triglycerides, leptin, and insulin) and metabolic protein expression patterns were evaluated.

Key findings: Lactulose treatment reduced adipogenesis and fat accumulation *in vitro* by down-regulation of major adipogenic transcription factors such as C/EBPα and PPARγ. *In vivo* treatment of lactulose to 5-week-old Sprague-Dawley male rats significantly alleviated HFD-induced body weight gain and food efficiency as well as improved plasma and other metabolic parameters. In addition, lactulose treatment down-regulated major adipogenic marker proteins (C/EBPα and PPARγ) in adipose tissue as well as stimulated expression of proteins involved in energy expenditure and lipolysis (ATP5B, COXIV, HSL, and CPT1).

Significance: In conclusion, reduced adipogenesis and increased energy expenditure mediated by lactulose treatment synergistically contribute to alleviation of HFD-induced body weight gain. Therefore, pharmaceutical targeting of GAL1 using lactulose would be a novel therapeutic approach for the treatment of obesity.

1. Introduction

Metabolic diseases such as type 2 diabetes, cardiovascular diseases, atherosclerosis, and cancer have been shown to be connected with obesity, which has emerged as a leading health hazard over recent decades [1]. Obesity and related metabolic complications are regulated through complex multi-protein networks, and these novel protein networks can be targeted for efficient obesity management [2,3]. Recently, multiple novel marker proteins from important obesity regulatory networks have been manipulated using specific inhibitors, and these inhibitors show great potential as anti-obesity drugs [4,5].

Galectins (GALs) are animal lectins with carbohydrate recognition domains (CRDs) and multiple functions in mammals [6]. Depending on their functions and molecular structures, GAL family proteins can be divided into 15 different types and are involved in various intra or extra cellular functions such as cell-cell adhesion and intracellular vesicle transport [7]. GAL1 is a prominent member of the GAL family, which contains a single CRD and forms symmetric homodimers using hydrophobic faces [8]. GAL1, an important endogenous member of the GAL family, is involved in regulation of the antigen-specific T cell response, apoptosis in thymocytes or activated T cells, as well as modulation of T cell receptor signaling through CD6 [9,10,11]. In connection with obesity, up-regulation of GAL1 has been observed in subcutaneous adipose tissues of obese patients and diet-induced obese mice models [12]. In addition, GAL1 is secreted at higher concentrations during adipocyte differentiation, demonstrating its involvement as a growth-modulating factor in adipocyte development [12,13]. Taken together, the adipocyte-specific roles of GAL1 led us to investigate its potential role as a positive regulator of adipogenesis and lipogenesis. Therefore, the current study was designed to identify a targeted inhibitor of GAL1 in order to determine whether or not pharmacological inhibition of GAL1 can alleviate body weight gain in a diet-induced obese animal model.

To date, several different types of glycoconjugates or synthetic chemicals have been identified based on their divergent binding capability towards GAL family proteins [14,15]. Lactulose and lactulose amines are small-sized inhibitors of GAL1 that are known to inhibit migration and invasion as well as induce apoptosis in endothelial cells and small cell lung cancer cells [14]. But, lactulose binding to different

* Corresponding author.
E-mail address: jwyun@daegu.ac.kr (J.W. Yun).

http://dx.doi.org/10.1016/j.lfs.2016.02.018
0024-3205/

members of galectin is dependent on thermodynamic interaction between galactose binding site and lactulose. This difference in interaction can affect specificity of lactulose to other members of galectin like GAL3, GAL12 [16]. Lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) is a semi-synthetic disaccharide comprised of fructose and galactose bonded together by a β-1,4-glycosidic bond, which is insensitive to mammalian digestive enzymes [17]. Due to its non-metabolizable characteristics, lactulose can readily pass through the stomach and small intestine, thereby providing a perfect medium for beneficial bacteria in the large intestine, especially members of bifidobacteria [18]. Oral administration of lactulose is already known to be beneficial for insulin resistance by improving insulin signaling and reducing free fatty acids as well as blood glucose levels in obese patients [19,20]. Intake of lactulose is a well-known medication for functional constipation [21] and has shown a beneficial impact on non-alcoholic steatohepatitis in rats when administered orally [22]. Colonic fermented products of lactulose are also known to reduce lipolysis in obese subjects by adjusting blood free fatty acid levels, improving free fatty acid-dependent insulin resistance [20]. In addition, lactulose-containing food preparations improve insulin signaling and have positive effects on blood glucose levels in obese patients [19]. However, no direct evidence has linked lactulose to anti-obesity effects until now.

The goal of this study was therefore to examine whether or not lactulose efficiently inhibits GAL1, thereby reducing adipogenesis and lipogenesis in both cultured white and brown adipocytes as well as diet-induced obese rats, in order to evaluate pharmaceutical targeting of GAL1 using lactulose as a novel therapeutic approach for treatment of obesity.

2. Materials and methods

2.1. Adipocyte culture and differentiation

3T3-L1 and HIB1B cells that derived from mice white and brown preadipocytes, respectively were cultured and differentiated using previously described protocols [23]. Briefly, adipocytes were cultured on appropriate plates until confluent, after which growth medium was changed to differentiation cocktail and maturation medium. Maturation medium was changed every 2 days. 3T3-L1 cell line was purchased from the Korean Cell Line Bank (KCLB10092.1), and HIB1B cell line was a kind gift from Dr. Kwang-Hee Bae [24]. Unless otherwise stated, both adipocytes were separately treated with 250 or 500 μM of lactulose in maturation medium for 4 days before further analysis. Cytotoxicity of lactulose on both adipocytes was assessed by MTT [3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Generay Biotech, Shanghai, China) using a previously described protocol [25].

2.2. Oil Red O staining and quantification of triglycerides (TG)

3T3-L1 and HIB1B adipocytes were matured for 4 days and used for Oil Red O staining as well as quantification of TG according to previously described methods [23]. Briefly, adipocytes were fixed with 10% formalin for 1 h at room temperature and stained by a mixture of Oil Red O and water at a 6:4 ratio for 10 min, followed by washing three times with deionized water. For TG quantification, mature cells were washed twice with PBS and harvested to prepare cell lysates using RIPA buffer (Sigma, St. Louis, MO, USA). TG content was measured according to the manufacturer's instructions using a TG test kit (Asan Pharm. Co., Yeongcheon, Korea), and absorbance was measured at 550 nm.

2.3. Quantitative real-time RT-PCR

Mature adipocytes were harvested and total RNA isolated using a RNA isolation kit (RNA-spin, iNtRON Biotechnology, Seongnam, Korea), after which 1 μg of RNA was converted into cDNA using Maxime RT premix (iNtRON Biotechnology). Transcription level of each gene was quantitatively determined by employing Fast start universal SYBR Green mastermix (Roche, Basel, Switzerland) with real-time RT-PCR (Stratagene 246 mx 3000p QPCR System, Agilent Technologies, Santa Clara, CA, USA) and normalized to the level of *β-actin*. Sequences of primer sets used in this study are listed in Table S1 in Supplementary Materials.

2.4. Immunoblot analysis

Lysates from both white adipose tissue (WAT) and brown adipose tissue (BAT) were prepared in RIPA Buffer (Sigma), followed by immunoblotting using monoclonal or polyclonal antibodies against GAL1, Peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer binding protein α (C/EBPα), Cytochrome c oxidase IV (COX IV), Hormone sensitive lipase (HSL), ATP synthase subunit beta, mitochondrial (ATP5B), Carnitine *O*-palmitoyltransferase 1 (CPT1), Uncoupling protein-1 (UCP1), and β-actin (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) according to common laboratory protocols [23]. Band intensities of target proteins were normalized using β-actin to calculate relative intensities of protein bands using ImageMaster software (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

2.5. Animal experiments

Five-week-old Sprague-Dawley (SD) male rats were acclimatized with normal chow for 1 week and then divided into four groups *viz.* control rats fed a normal diet (NC), HFD-fed control rats (HC), HFD-fed rats treated with lactulose (LT) by oral administration (LT-OR), and HFD-fed rats treated with lactulose by intraperitoneal injection (LT-IP). PBS or lactulose solubilized in PBS was administered either by oral gavage or intraperitoneal injection every 4th day to rats at a dose of 5 mg/kg body weight for 8 weeks. ND and HFD groups contained 12% and 60% fat as an energy source, respectively (Korea Lab., Hanam, Korea). All animal experiments were approved by the Committee for Laboratory Animal Care and Use of Daegu University.

2.6. Quantification of plasma TG, FFA, leptin, AST, and ALT

Blood samples were collected from abdominal aortas and collected into EDTA tubes (BD, Franklin Lakes, NJ, USA) to isolate plasma by centrifugation (3000×*g*, 10 min), followed by storage at −80 °C until further analysis. Common plasma biochemical characterizations were performed using commercial ELISA kits for AST-ALT (Asan Pharm. Co., Seoul, Korea), cholesterol (Biovision Inc. Milpitas, CA, USA), FFA, leptin (Enzo Life Sciences Inc., Farmingdale, NY, USA), plasma TG (Cayman Chemical Company, Ann Arbor, MI, USA), free glycerol (Sigma), and insulin (ALPCO, Salem, NH, USA). All ELISA experiments were carried out in triplicate using individual plasma samples to quantify plasma parameters.

2.7. Statistical analysis

Results are expressed as the mean ± S.D. Statistical significances were calculated using one-way (0 μM vs. 250, 500 μM of lactulose) or two-way ANOVA (HC vs. NC, LT-OP, LT-OR), followed by Tukey's post-hoc test (for more than two groups), where $p < 0.05$ or $p < 0.01$.

3. Results

3.1. Lactulose treatment reduces lipid content and lipogenic markers in cultured adipocytes

Lactulose treatment attenuated TG content in both 3T3-L1 and HIB1B adipocytes, and Oil Red O staining also demonstrated reduced oil droplet accumulation in lactulose-treated adipocytes (Fig. 1A, B).