NUTRITION RESEARCH 35 (2015) 317–327

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Original Research

Myricetin suppresses differentiation of 3 T3-L1 preadipocytes and enhances lipolysis in adipocytes

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ARTICLE INFO

Article history:
Received 19 September 2014
Revised 29 December 2014
Accepted 30 December 2014

Keywords:
Myricetin
3 T3-L1 adipocyte
Differentiation
Lipolysis
MAPK

ABSTRACT

Myricetin (MyR), a naturally occurring flavonol widely distributed in fruits, vegetables, and medicinal plants, has anticancer, anti-inflammatory, antihyperlipidaemic, and antiobesity activities. In the present study, we hypothesized that the antiobesity property of MyR is mediated via suppression of differentiation of preadipocytes into adipocytes and promotion of lipolysis of mature adipocytes, which effectively decrease the intracellular triglyceride concentration of adipocytes. Accordingly, the aim of this work was to investigate the effects of MyR on adipocyte differentiation and lipolysis in differentiated 3 T3-L1 adipocytes. Our results showed that MyR inhibited differentiation of 3 T3-L1 preadipocytes in a concentration-dependent manner. Myricetin downregulated the mRNA and protein levels of CCAAT/enhancer-binding protein α and peroxisome proliferator-activated receptor γ, both of which are major adipogenic transcription factors. Furthermore, the mRNA levels of other adipogenesis-related transcription factors, namely, CCAAT/enhancer-binding protein β, sterin regulatory element binding protein 1-c, peroxisome proliferator-activated receptor γ coactivator–1, adipocyte protein 2, lipoprotein lipase and glucose transporter 4, were also reduced by MyR treatment. Moreover, MyR significantly inhibited the phosphorylation of extracellular signal–regulated kinase, Jun N-terminal kinase, and p38 during the differentiation process. On the other hand, MyR induced a dose-dependent increase in glycerol release in fully differentiated adipocytes, indicating its stimulatory effect on adipocyte lipolysis. Furthermore, MyR downregulated mRNA level of perilipin A and enhanced the phosphorylation level of extracellular signal–regulated kinase, Jun N-terminal kinase, and p38 during lipolysis. Taken together, these findings indicate that MyR exerts antiobesity activity in adipocytes.

Abbreviations: ANOVA, analysis of variance; aP2, adipocyte protein 2; ATGL, adipose triglyceride lipase; BSA, bovine serum albumin; C/EBPs, CCAAT/enhancer-binding proteins; DMEM, Dulbecco modified Eagle medium; DMSO, dimethyl sulfoxide; ERK, extracellular signal–regulated kinase; FBS, fetal bovine serum; GLUT 4, glucose transporter 4; HSL, hormone-sensitive lipase; JNK, Jun N-terminal kinase; LPL, lipoprotein lipase; MAPK, mitogen-activated protein kinase; PGC-1, peroxisome proliferator-activated receptor γ coactivator–1; PPAR γ, peroxisome proliferator-activated receptor γ; q-PCR, real-time quantitative reverse transcriptase polymerase chain reaction; SRB, sulforhodamine B; SREBP1-c, sterin regulatory element binding protein 1-c; TG, triglyceride.

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http://dx.doi.org/10.1016/j.nutres.2014.12.009
0271-5317/

1. Introduction

Obesity has become a global epidemic and a worldwide public health problem in recent years [1]. Obesity is characterized by elevated fat mass, namely, increase in both adipocyte number and size [2]. Intracellular triglyceride (TG) concentration of adipocytes, which increases during differentiation of preadipocytes into adipocytes and decreases during lipolysis of adipocytes [3,4], is another useful index for obesity. Hence, reducing fat by suppressing differentiation of preadipocytes and promoting lipolysis of adipocytes have been proposed as antiobesity measures. Numerous studies [3–6] have been carried out to investigate the mechanisms underlying these processes to facilitate obesity treatment. Peroxisome proliferator-activated receptor γ (PPAR γ) and CCAAT/enhancer-binding protein (C/EBPs), known as major transcription factors, have been proposed to play important roles during the preadipocyte differentiation process [7–9]. Sterin regulatory element binding protein 1-c (SREBP1-c), peroxisome proliferator-activated receptor γ coactivator–1 (PGC-1), glucose transporter 4 (GLUT 4), adipocyte protein 2 (aP2), lipoprotein lipase (LPL), and other differentiation related genes are also involved in the process [10–12]. Moreover, the mitogen-activated protein kinase (MAPK) signaling pathway regulates the expression of PPAR γ and C/EBP α mRNA during differentiation of 3 T3-L1 preadipocytes [13]. By contrast, during adipocyte lipolysis, adipose triglyceride lipase (ATGL) cleaves triglycerides, whereas another adipose lipase called *hormone-sensitive lipase* (HSL) displays higher specific activity against diglycerides [14,15]. Lipoprotein lipase is another major enzyme responsible for hydrolysis of TG molecules present in circulating lipoproteins [16]. However, lipase is not the sole mediator of stimulated lipolysis. Perilipin A, the proteinaceous coating of the lipid storage droplet, has a central role in lipid metabolism and adipocyte lipolysis; lipolysis cannot proceed normally without it [17]. Additionally, the MAPK signaling pathway, specifically extracellular signal–regulated kinase (ERK), is also involved in the regulation of lipolysis [18].

Recent studies have focused on discovering plant-derived antiobesity molecules that have minimal adverse effects and can be easily obtained. Myricetin (3, 5, 7, 3′,4′,5′-hexahydroxyflavone; MyR), one of the main compositions of flavonols, is commonly found in tea, berries, fruits, vegetables, and some medicinal herbs [19,20] and has been demonstrated to have potential for treatment of diabetes [20,21], cancer [22], inflammation [23], malaria [24], and amyloid-β–related neuropathogenesis [25]. Additionally, MyR has been found to exhibit antihyperlipidemic and antiobesity activities in high-fat diet–fed rats [26]. Myricetin has also been reported to inhibit adipogenesis during differentiation of 3 T3-L1 preadipocytes [26–28] by decreasing mRNA levels of PPAR γ, CEBP/α, and ap2 [28]. However, the mechanism by which MyR downregulates these major adipogenic transcription factors remains unresolved. In contrast to the extensive studies focusing on the effect of MyR on adipocyte differentiation, information regarding the effect of MyR on lipolysis, another important pathway for suppressing fat accumulation, is scarce.

In the present study, we hypothesized that the antiobesity property of MyR is mediated by suppressing differentiation of preadipocytes into adipocytes and promoting lipolysis of mature adipocytes, which consequently decrease the intracellular triglyceride concentration of adipocytes. To test this hypothesis, we investigated the effect of MyR on preadipocyte differentiation and adipocyte lipolysis. Differentiation of adipocytes was evaluated based on cellular accumulation of lipid droplets, and the modulatory mechanism was tested by measuring expression levels of adipocyte-specific transcription factors and phosphorylation of MAPK. In addition, the effect of MyR on adipocyte lipolysis was investigated by analyzing glycerol release and gene expression.

2. Methods and materials

2.1. Materials

MyR, 3-isobutyl-1-methylxanthine, and indomethacin were purchased from Aladdin Inc (Shanghai, China). Insulin, sulforhodamine B sodium salt (SRB), Oil Red O, and dimethyl sulfoxide (DMSO) were purchased from Sigma (St Louis, MO, USA). Dexamethasone was purchased from ACORS (Lauro Linhares, Brazil). Myricetin was reconstituted in DMSO and stored at –20°C until use.

2.2. Cell culture and differentiation

3 T3-L1 mouse fibroblast cells were obtained from The National Center for Drug Screening (Shanghai, China) and cultured following the procedure of Drira and Sakamoto [5] with some modifications. Fibroblast cells were cultured in high-glucose Dulbecco modified Eagle medium (DMEM; Gibco, CA, USA) supplemented with 10% newborn calf serum (NCS; Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco) at 37°C under a 5% CO_2 atmosphere. Confluent 3 T3-L1 cells were maintained in culture medium for 2 days (day 0) to induce differentiation. Cells were exposed to differentiation medium 2 (DMEM containing 10 μg/mL insulin, 0.5 μmol/L 3-isobutyl-1-methylxanthine, 1 μmol/L dexamethasone, 0.2 mmol/L indomethacin, and 10% fetal bovine serum [FBS; Hyclone, Logan, UT]) for 3 days (day 3). The medium was then changed with differentiation medium 1 (DMEM containing 10 μg/mL insulin and 10% FBS) for an additional 3 days (day 6). Subsequently, the cells were maintained in DMEM containing 10% FBS for an additional 2 days (day 8). Afterward, the differentiated adipocytes were harvested for further experiments.

Varying concentrations of MyR (0, 0.1, 1, 10, 50, and 100 μmol/L) were added to the medium throughout the differentiation period (from days 0 to 8) to observe effects on adipogenesis during differentiation.

Differentiated 3 T3-L1 adipocytes under normal conditions were incubated in phenol red-free DMEM containing 0.2% bovine serum albumin (BSA; MP, Santa Ana, CA) in the absence of serum for 12 hours to investigate the effect of MyR on lipolysis. Afterward, the DMEM was removed and replaced with the same DMEM containing various concentrations of MyR.

2.3. SRB assay

Sulforhodamine B protein stain assay was used for the measurement of cell proliferation. The 3 T3-L1 preadipocytes