Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Stereoselective effects of lactate enantiomers on the enhancement of 3T3-L1 adipocyte differentiation

Naoki Harada [a, *], Ito Hirano [a], Hiroshi Inui [b], Ryoichi Yamaji [a]

[a] Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka, Japan
[b] Division of Clinical Nutrition, Graduate School of Comprehensive Rehabilitation, Osaka Prefecture University, Habikino, Osaka, Japan

ARTICLE INFO

Article history:
Received 23 February 2018
Accepted 27 February 2018
Available online xxx

Keywords:
Lactic acid
Enantiomer
Enantioselectivity
Adipocyte differentiation
3T3-L1 preadipocyte

ABSTRACT

Lactate contains a chiral carbon and thus has two optical isomers—D-lactate and L-lactate. L-Lactate is the predominant form that is produced by the body and can be delivered to the organs. On the other hand, gut microbiota produce both isomers, which can then flow into the body. Although both D-lactate and L-lactate can contribute to energy metabolism, their potential roles in adipocyte differentiation remain to be elucidated. Here, we investigated the effects of L-lactate and D-lactate on the differentiation of 3T3-L1 preadipocytes. Both lactate enantiomers were demonstrated to enhance triglyceride accumulation by stimulating the early phase of adipocyte differentiation. Notably, D-lactate was more potent than L-lactate in inducing triglyceride accumulation. The degree of triglyceride accumulation induced by L-lactate was similar to that induced by pyruvate. D-Lactate was more potent than L-lactate in increasing the activity of glycerol-3-phosphate dehydrogenase. Both lactate enantiomers did not affect cell viability. Moreover, both enantiomers upregulated the expression of peroxisome proliferator-activated receptor γ, CCAAT/enhancer-binding protein (C/EBP) α, sterol regulatory element-binding protein-1c, and fatty acid synthase, with D-lactate exerting stronger effects than L-lactate. By contrast, lactate did not influence the expression of C/EBPβ and C/EBPδ. D-Lactate significantly increased and L-lactate tended to increase p38 MAPK phosphorylation, and the p38 MAPK inhibitor SB203580 inhibited the stimulation of adipocyte differentiation by D-lactate and L-lactate. These findings showed that both lactate enantiomers stimulate preadipocyte differentiation, with D-lactate showing more potent effects than L-lactate. In addition, our study demonstrated that D-lactate and L-lactate exert different effects on physiological events.

1. Introduction

Lactate is the simplest hydroxycarboxylic acid that exhibits structural chirality and consists of two optical isomers, namely, D-lactate and L-lactate. Both enantiomers are present in the human body. L-Lactate is the major form of lactate in the blood of healthy human subjects and is usually present at the low millimolar range (~1–2 mM), whereas D-lactate exists at the nanomolar range (below 100 nM) [1]. L-Lactate is produced from pyruvate by L-lactate dehydrogenase, whereas D-lactate is produced via methylglyoxal metabolism in humans [1]. Blood L-lactate concentrations are elevated during anaerobic exercise. After heavy physical exercise, blood lactate concentrations can increase to 10 mM [2,3]. On the other hand, plasma D-lactate concentrations also can reach up to 10 mM in subjects with gastrointestinal disorders [4,5]. The increase in D-lactate concentrations is caused by the influx of both lactate enantiomers that are extensively produced by gut microbiota in the intestinal tract [1].

Obesity is a major health concern worldwide [6]. Excess energy intake results in an enlargement of adipose tissue mass, which is determined by both adipocyte volume (*i.e.*, hypertrophy) and adipocyte number (*i.e.*, adipogenesis and adipose cell death) [7]. Adipogenesis is regulated by both hyperplasia and preadipocyte differentiation [8,9], and adipocytes accumulate triglycerides as a storable energy source. 3T3-L1 preadipocyte cells are the most established model for studying differentiation from preadipocytes to adipocytes [10,11]. Preadipocyte differentiation is classified into

Abbreviations: cAMP-response element, CRE; CCAAT/enhancer-binding protein, C/EBP; dexamethasone, DEX; glycerol-3-phosphate dehydrogenase, GPDH; fatty acid synthase, FAS; 3-isobutyl-1-methylxanthine, IBMX; peroxisome proliferator-activated receptor, PPAR.

* Corresponding author. Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan.
E-mail address: harada@biochem.osakafu-u.ac.jp (N. Harada).

https://doi.org/10.1016/j.bbrc.2018.02.198
0006-291X/

three stages, namely, the early, middle, and late stages, and is regulated by specific transcription factors [10]. CCAAT/enhancer-binding protein β (C/EBPβ) and peroxisome proliferator-activated receptor γ (PPARγ) are the master regulatory transcription factors that are active during the early and middle to late stages of adipocyte differentiation, respectively [10–12]. C/EBPδ and C/EBPα are also crucial transcription factors involved in the early and middle to late stages of differentiation, respectively [10,11]. Stimulation of PPARγ and C/EBPα activates fatty acid synthase (FAS), which contributes to fatty acid biosynthesis [10,11]. In addition to fatty acids, glycerol 3-phosphate is required for triglyceride synthesis, and the activity of glycerol-3-phosphate dehydrogenase (GPDH) has been used as an adipocyte differentiation marker [13]. Adipocyte differentiation is initiated by approximately two rounds of cell division in a process called mitotic clonal expansion [14].

Recently, blood L-lactate, not glucose, was identified as the primary carbon source of the tricarboxylic acid cycle in most tissues, including adipose tissue [15]. On the other hand, lactate has been reported to suppress noradrenalin-induced lipolysis *in vitro* [16], suggesting its role as a signaling molecule. In addition, the effects of lactate on adipogenesis ramain unclear. Lactate is believed to predominantly exist in the L-enantiomer form because of its abundance in the blood of healthy subjects. However, D-lactate concentrations can reach the millimolar range [2–5], and the differences in the physiological effects of the lactate enantiomers remain to be investigated. In the present study, we demonstrated that L-lactate and D-lactate enhance preadipocyte differentiation but exert different effects.

2. Materials and methods

2.1. Cell culture

3T3-L1 murine preadipocytes were obtained from the Japanese Collection of Research Bioresources and cultured as previously described [17]. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 4.5 g/L glucose, 10% bovine serum (16170-078, Life Technologies), and antibiotics (100 U/mL penicillin G and 100 μg/mL streptomycin sulfate). Cells were maintained at 37 °C with 5% CO_2/95% air atmosphere and 98% humidity. Adipocyte differentiation of 3T3-L1 preadipocytes was induced as follows. At 2 days post-confluency (day 0), the culture medium was replaced with differentiation medium containing 4.5 g/L glucose, 10% fetal bovine serum, and antibiotics. Differentiation was induced by the addition of 1 μM dexamethasone (DEX), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 10 μg/mL bovine insulin. Next, the culture medium was replaced every 2 days with fresh differentiation medium containing 10 μg/mL bovine insulin, and the cells were differentiated for a total of 8 days. Control cells (without differentiation) were not added with the differentiation stimuli (*viz.*, DEX, IBMX, insulin). Cells were treated with sodium D-lactate, sodium L-lactate, sodium pyruvate, or NaCl each time the culture medium was replaced (days 0, 2, 4, and 6).

2.2. Oil red O staining and measurement of triglyceride levels and GPDH activity

Cells were stained with Oil red O [17] and analyzed using a microscope (Nikon TMS, Nikon) equipped with WRAYCAM NF300 (WRAYCAM). Triglyceride levels were determined as previously described [17] using a Triglyceride E-test Wako (Wako Pure Chemical Industries). GPDH activity was measured at 37 °C as previously described [17].

2.3. Cell viability assay

3T3-L1 cells were plated onto a 48-well plate. After reaching confluence, cells were incubated with sodium lactate or sodium pyruvate for 8 days under differentiation conditions. Medium containing sodium lactate or sodium pyruvate was replaced with fresh medium every 2 days. Cell viability was measured using AlamarBlue (Trek Diagnostics Systems), and fluorescence was determined as previously described [18].

2.4. Western blotting

Cells were harvested, and cell lysates were prepared as previously described [17]. The lysates were resolved via SDS-PAGE, followed by western blotting using polyclonal anti-total or polyclonal anti-phosphorylated p38 MAPK antibody (Cell Signaling Technology). Membranes were then incubated with horseradish

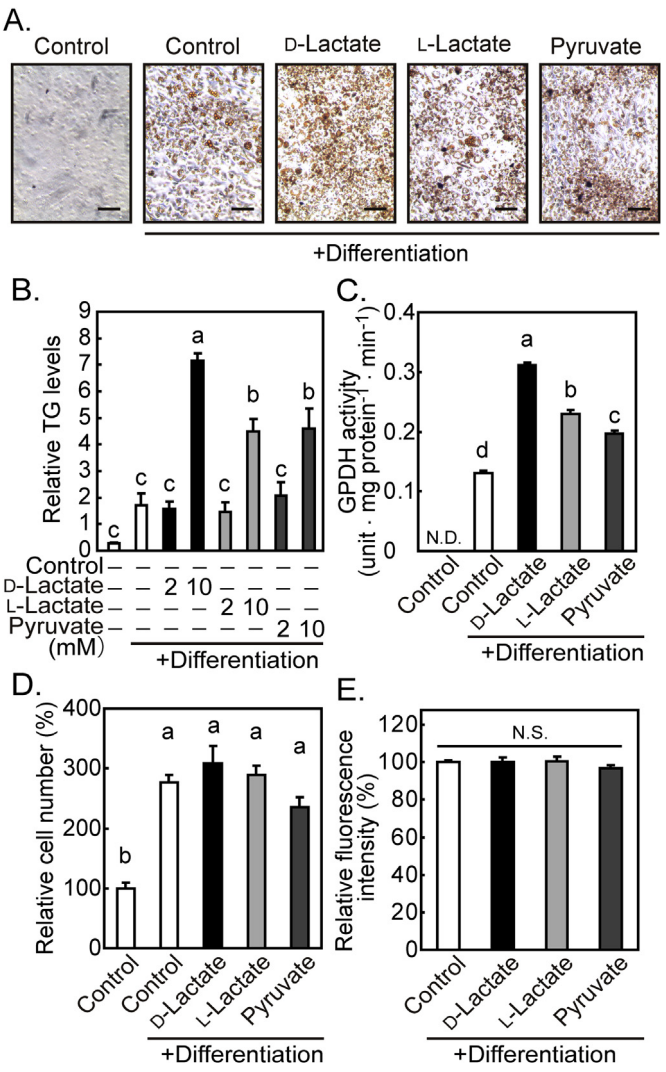

Fig. 1. Effects of D- and L-lactate on the differentiation of 3T3-L1 preadipocytes. 3T3-L1 cells were differentiated in the presence of 10 mM sodium lactate or sodium pyruvate. NaCl (10 mM) was used as control. (A) Oil red O-staining on day 8. Scale bar indicates 100 μm. (B) Triglyceride (TG) levels per dish on day 8. (C) Glycerol-3-phosphate dehydrogenase (GPDH) activity on day 8. (D) Cell counts of 3T3-L1 cells on day 2. (E) Cell viability determined using Alamar Blue dye on day 8. Data are expressed as mean ± SEM (n = 3 for B; n = 4 for C and E; n = 6 for D). Different letters indicate statistically significant differences ($p < 0.05$). N.D.: not detected. N.S.: not significant.

Please cite this article in press as: N. Harada, et al., Stereoselective effects of lactate enantiomers on the enhancement of 3T3-L1 adipocyte differentiation, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.02.198