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A new hope for obesity management: Boron inhibits adipogenesis in progenitor cells through the Wnt/β-catenin pathway

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ABSTRACT

Obesity is a worldwide medical problem resulting in serious morbidity and mortality involving differentiation of pre-adipocytes into mature adipocytes (adipogenesis). Boron treatment has been reported to be associated with weight reduction in experimental animals; however, its effects on pre-adipocyte differentiation and anti-adipogenic molecular mechanisms are unknown.

In this study, we demonstrate the inhibitory activities of boric acid (BA) and sodium pentaborate pentahydrate (NaB) on adipogenesis using common cellular models. Boron treatment repressed the expression of adipogenesis-related genes and proteins, including CCAAT-enhancer-binding protein α and peroxisome proliferator-activated receptor γ, by regulating critical growth factors and the β-catenin, AKT, and extracellular signal-regulated kinase signaling pathways. In addition, although boron treatment did not induce apoptosis in pre-adipocytes, it depressed mitotic clonal expansion by regulation of cell cycle genes.

Overall, these data offer promising insights into the prevention/treatment of obesity and associated diseases.

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1. Introduction

Obesity and associated comorbidities, such as type 2 diabetes, hypertension, cardiovascular disease, cancer, and metabolic syndrome or posttraumatic stress disorder (PTSD), are worldwide health concerns, particularly in developed countries, leading to increased healthcare costs, morbidity, and mortality [1,2]. An estimated 2.1 billion people worldwide are thought to be overweight or obese, and 2.8 million deaths are associated with obesity annually [3]. Although the exact molecular mechanisms of obesity are not well understood, uncontrolled hyperplasia and hypertrophy of adipocytes, the main units in fat tissue, are

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known to contribute to the development of obesity [4]. Adipogenesis is triggered by a cascade of transcriptional regulators, mainly peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT-enhancer-binding proteins (C/EBPs), along with regulation of several signaling pathways, including Wnt/β-catenin and AKT [5–7]. In the canonical Wnt pathway, stabilized β-catenin translocates into the nucleus and binds to T cell factor/lymphoid-enhancing factor (TCF/LEF) family of transcription factors, which subsequently inhibits expression of adipogenesis related transcriptional factors [8]. The main approaches to treating obesity focus on targeting these pathways to limit lipid storage and adipocyte cell propagation. Although some therapeutic options have been presented to the market for the treatment of obesity, their clinical use is limited due to having severe side effects including hypertension, cardiovascular problems, liver diseases and psychiatric disorders [9]. Development of new, safe and efficient alternatives are therefore required.

Boron, a naturally occurring element, is found in various human tissues. However, the importance and necessity of boron have not yet been fully elucidated. Boron is involved in several metabolic pathways, including wound healing [10], bone development and maintenance [11], hormone [12] and psychological activities [13]. Recent studies have shown that usage of boron as a dietary supplement results in short- and long-term weight loss [14,15]. In a previous study, chicks fed boron (3 mg/kg) showed moderate weight loss and decreased plasma glucose levels, likely owing to magnesium and vitamin D3 deficiency [16]. In another work, severe body weight reduction was observed in male rats treated with boron compounds for 90 days [17]. Although limited numbers of reports have shown the weight loss inducing effect of boron derivatives, the precise mechanism of action behind boron mediated weight loss is unclear.

In this study, we aimed to elucidate the effects of boron derivatives on adipogenesis in pre-adipocytes and pluripotent cells by using in vitro models. To this end, C3H10T1/2 pluripotent cells, 3T3-L1 and 3T3-F44 preadipocytes, widely used cell lines in in vitro adipogenesis researches [18], were differentiated in the presence of boron compounds.

2. Materials and Methods

2.1. Materials

BA (#10043–35-3) was purchased from Bio Basic (Canada), and NaB was provided by the National Boron Research Institute-BOREN (Ankara, Turkey). Dulbecco's modified Eagle medium (DMEM), fetal calf serum (FCS), fetal bovine serum (FBS), and penicillin–streptomycin–amphotericin (PSA) were obtained from ThermoFisher. All other cell culture reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. All experiments were repeated at least three times. The experimental procedures are explained briefly below; more detailed methodologies can be found in the Supplemental Experimental Procedures.

2.2. Cell Lines and Differentiation

The pre-adipocyte cell lines 3T3-L1 (CL-173; ATCC, Manassas, VA) and 3T3-F442A (EF3002; Kerafast) were cultured in DMEM containing 10% FCS and 1% PSA. The cells were differentiated as described previously [19,20]. Murine pluripotent C3H10T1/2 cells (CCL-226; ATCC) were maintained in DMEM containing 10% FBS and 1% PSA and were differentiated as previously reported [21]. After adipogenic differentiation, cells were fixed with 4% paraformaldehyde for 45 min at room temperature, and Oil Red O staining was performed to stain lipid droplets [22]. Spectrophotometric quantification of lipid staining was performed by measuring the optical density of isopropanol-extracted Oil Red O dye at 490 nm.

2.3. Cytotoxicity Assay

The effects of BA and NaB on adipocyte proliferation and relative cell numbers at the end of differentiation were investigated using MTS assays (CellTiter96 Aqueous One Solution; Promega, Southampton, UK).

2.4. Triglyceride Quantification

The triglyceride contents of differentiated and undifferentiated cells were evaluated using a Triglyceride Colorimetric Assay Kit (10,010,303; Cayman Chemicals, Canada) according to the manufacturer's instructions.

2.5. Apoptosis and Cell Cycle Analysis

Apoptotic and necrotic cell fractions in boron-treated cells were determined using an Annexin V kit (sc-4252 AK; Santa Cruz Biotechnology, Santa Cruz, CA). The cell cycle distribution after boron treatment was measured using propidium iodide staining. All apoptosis and cell cycle analyses were performed using a Becton Dickinson FACSCalibur flow cytometry system (Becton Dickinson, San Jose, CA).

2.6. Immunocytochemical Analysis

Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton-X 100/phosphate-buffered saline (PBS) solution followed by blocking with 1% bovine serum albumin (BSA). Cells were incubated overnight at 4 °C with the following primary antibodies: anti-β-actin (#3700; Cell Signaling Technology, Danvers, MA), anti-PPARγ (MA5–14889; Sigma), anti-CEBPα (#8178; Cell Signaling Technology), anti-FABP4 (sc-271,529; Santa Cruz Biotechnology), and anti-β-catenin (#8814; Cell Signaling Technology). Then, cells were treated with AlexaFluor 488- or 647-conjugated goat anti-rabbit or anti-mouse IgG (ThermoFisher) for 1 h at room temperature. Nuclei were stained with DAPI (Sigma), and samples were visualized with a confocal microscope (LSM 700; Zeiss, Heidelberg, Germany).

2.7. BODIPY Staining

Differentiated 3T3-L1 cells were stained with 5 μg/mL BODIPY 493/503 (ThermoFisher) for 15 min at 37 °C and washed four times with ice-cold PBS containing 0.1% BSA. The samples were visualized using confocal microscopy.

2.8. Reverse Transcription Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was isolated from experimental groups using TRIzol Reagent (ThermoFisher), and cDNAs were synthesized