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ACE2 activator diminazene aceturate reduces adiposity but preserves lean mass in young and old rats

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ABSTRACT

The obesity epidemic is multi-generational and is particularly debilitating in the aging population, necessitating the use of pharmaceutical interventions. Recent evidence suggests that increasing the activity of the angiotensin converting enzyme-2 [ACE2]/angiotensin-(1-7)[Ang-(1-7)]/Mas receptor (MasR) axis in obese animal models leads to significant reductions in body weight. It was hypothesized that activation of ACE2 via diminazene aceturate (DIZE) will significantly reduce body weight of rats fed a high fat diet. Young and old (4 and 23 months, respectively) male Fisher 344 × Brown Norway rats were fed 60% high fat diet for one week, and subsequently given either 15 mg/kg/day DIZE s.c. or vehicle for three weeks. DIZE treatment resulted in a significant reduction of food intake and body weight in both young and old animals. However, that decrease was so dramatic in the older animals that they all nearly stopped eating. Interestingly, the TD-NMR assessments revealed that the weight-loss was primarily a result of decreased body fat percentage, with a relative preservation of lean mass. Tissue weights confirm the significant loss of white adipose tissue (WAT), with no change in muscle weights. Gene expression and serum ACE2 activity analyses implied that increased activation of the ACE2/Ang-(1-7)/MasR axis plays a role in reducing fat mass. Collectively, our results suggest that DIZE may be a useful tool in the study of obesity; however, caution is recommended when using this compound in older animals due to severe anorectic effects, although there is a mechanism by which muscle is preserved.

1. Introduction

Obesity is a global epidemic affecting the health of both young and old generations (Zamboni and Mazzali, 2012; Ogden et al., 2014). Current recommendations for obesity treatment include adherence to a healthy diet and increased physical activity. Unfortunately, many patients, including the ever-growing aging-obese population, are physically unable or unwilling to adhere to these recommendations (Zamboni and Mazzali, 2012). For such individuals, novel strategies must be implemented to induce weight-loss and reduce the risk of associated co-morbidities, such as type 2 diabetes and cardiovascular disease. To this end, recent evidence suggests treatment with Angiotensin-(1-7) [Ang-(1-7)], a component of the renin angiotensin system (RAS), improves metabolism, glucose homeostasis, and lipid profile in animal models of obesity and metabolic syndrome (Rubio-Ruiz et al., 2014; Feltenberger et al., 2013). Furthermore, Ang-(1-7) treatment leads to reduced inflammation and oxidative stress, two key risk factors in the development of hypertension. These actions are in direct opposition to those of Ang II, the primary effector of the RAS, at the angiotensin type 1 receptor (AT_1R) (Simoes et al., 2013; Iusuf et al., 2008; Kostenis et al., 2005). Ang-(1-7) is a derivative of Ang II, formed when ACE2 cleaves the amino acid, phenylalanine, from the C-terminus (Zisman et al., 2003). As a consequence, increasing ACE2 activity has a two-fold effect: 1) it elevates the levels of the beneficial Ang-(1-7), while 2) decreasing the detrimental effects of Ang II. This study tests the hypothesis that increasing ACE2 activity will exert beneficial metabolic actions that thwart high-fat diet-induced obesity in both young and aged rats.

To test this hypothesis, we utilized diminazene aceturate (DIZE), a compound that is commonly used in livestock to treat Trypanosomiasis,

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or African sleeping sickness (Peregrine and Mamman, 1993) and that has more recently, come to be recognized for its ability to increase ACE2 activity in vitro and in vivo (Shenoy et al., 2013). Due to the ACE2 activating properties of this drug, recent studies have evaluated its potential as a pharmacological intervention in multiple rodent models of cardiovascular disease and each of these studies have revealed novel roles of increased ACE2 activity (Shenoy et al., 2013; Qi et al., 2013a; Bennion et al., 2015). Of particular relevance for the present studies, de Macedo et al. demonstrated a decrease in lipogenic enzymes in adipose tissue of mice treated with DIZE (de Macedo et al., 2015). Here, we assess the efficacy of this drug to prevent diet-induced obesity in both young and aged rats and also its impact on indices of ACE2/Ang-(1-7)/MasR axis activation in serum and tissue homogenates of these animals.

2. Materials and methods

2.1. Experimental animals

Three month-old and 22 month-old male Fisher 334 X Brown Norway rats were obtained from National Institute on Aging. Upon arrival, rats were examined and remained in quarantine for one week. Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals, and the University of Florida Institutional Animal Care and Use Committee approved all protocols. Rats were housed individually on a 12:12 h light-dark cycle and were fed standard chow for one month before the start of the experiment, whereupon they were fed 60% High Fat Diet (HF) (60% kcal from fat, 20% kcal from protein, 20% kcal from carbohydrates; Research Diets Inc., New Brunswick, NJ, USA).

2.2. Experimental design

Eight days after the start of HF, rats were pseudo-randomized into four groups (Young Control, n = 6; Young DIZE, n = 6; Old Control, n = 8; Old DIZE, n = 9) based on body weight to ensure that rats of various weights were represented equally in each group, and given either 15 mg/kg/day DIZE (LKT Laboratories Inc.; St. Paul, MN) or vehicle (water) s.c. Body weight and food intake were measured daily during the first week to document the hyperphagic response to the introduction of the HF diet and then subsequently measured twice weekly. Food and water were provided ad libitum in a food hopper that rested inside the cage above the animal. Daily food intake was measured by placing all food pellets remaining in the hopper on the scale. Body composition was measured at weeks 1 and 3 after treatment began via time-domain nuclear magnetic resonance (TD-NMR) in restrained but fully conscious rats (TD-NMR Minispec, Bruker Optics, The Woodlands, TX, USA). Treatment lasted for three weeks, and animals were sacrificed 24 h after final DIZE injection.

2.3. Tissue harvest

Rats were euthanized by thoracotomy under 5% isoflurane anesthetic. Whole blood was taken by cardiac puncture and serum collected following centrifugation in serum separator tubes. Subsequently, 15 ml of cold saline were perfused through the circulatory system. The perirenal, retroperitoneal, and epididymal white adipose depots (PWAT, RTWAT, and EWAT, respectively) along with interscapular brown adipose tissue (BAT), tibialis anterior (TA), and heart were excised, blotted dry, and weighed. The tibia was collected and used as a measurement of rat growth.

2.4. Serum ACE2 activity and leptin levels

Serum ACE2 activity was determined using the protocol described by Bennion et al. (2015). Briefly, serum samples (6 µl) were incubated in black flat-bottomed 96-well plates in 100 µl of reaction mixture containing ACE2 buffer (1 mol/L NaCl, 75 mmol/L Tris HCl, ph 7.5, and 50 µmol/L ZnCl2), 10 µmol/L captopril, and 25 µmol/L fluorogenic Mca-YVADAPK(Dnp)-OH ACE2 substrate (R&D Systems, Inc., #ES007). Relative fluorescence (RFU) for all samples was measured for 120 min using a Synergy Mx Microplate Reader (BioTek Instruments, Inc.) with excitation at 320 nm and emission at 405 nm. The slope of the fluorescence curve from 30 to 60 min was used to calculate RFU per minute. Substrate concentrations were selected following determination of reaction Km and Vmax using control samples and recombinant human ACE2 (R&D Systems, Inc., #933-ZN-010) as a positive control, and all samples were run in duplicate. Serum leptin levels were determined by ELISA (Millipore, Billerica, MA).

2.5. mRNA Gene Expression via qPCR

RTWAT (100 mg) and TA muscle (50 mg) was placed in 1 mL TriReagent (Sigma-Aldrich, St. Louis, Mo) and briefly sonicated. mRNA was isolated following standard TriReagent protocol from Sigma. mRNA was converted into cDNA using Applied Biosystems High Capacity cDNA Reverse Transcription Kit (Life Technologies, Grand Isle, NY). Real-Time PCR was used to determine gene expression of AT_1R, AT_2R, MasR, ACE, and ACE2 using TaqMan probes and the One-Step PCR machine (Life Technologies, Grand Isle, NY).

2.6. Western analysis

Brown adipose tissue (BAT; 30 mg) was briefly sonicated in 300 µl 10 mM Tris, pH 6.8, 2% SDS for Western analysis. BAT homogenate was diluted 1:30 (10 µl in 290 µl buffer) and filtered through 45 µm syringe filter before protein assay. Protein was determined by DC Bradford assay (Bio-Rad, Hercules, CA). Protein homogenate was separated on a SDS-PAGE gel and electro-transferred to nitrocellulose membranes. Immunoreactivity was assessed with antibodies specific to UCP-1 (Abcam, Cambridge, MA), and was detected with ECL prime (GE Healthcare, Piscataway NJ), scanned with a ChemiDoc XRS + (BioRad, Hercules, CA), and quantified using ImageQuant software (Molecular Dynamics, GE Healthcare Bio-Sciences, Pittsburgh, PA).

2.7. Statistical analysis

Data were analyzed by two-way ANOVA with repeated measures where appropriate, with Bonferroni post-hoc tests, using GraphPad Prism software (La Jolla, CA). Variables assessed in the analysis include age (Young vs. Old), Treatment (Control vs. DIZE), and Time. Values were considered statistically significant when $p < 0.05$.

3. Results

3.1. Effect of DIZE on food intake and body weight

DIZE treatment resulted in a significant reduction of caloric intake followed closely by a change in body weight in both young and old animals (Fig. 1). Results from repeated measures two-way ANOVA on kcal intake showed a significant main effect of treatment and age ($p < 0.0001$; $F(3,24) = 55.79$), and time ($p < 0.0001$; $F(15,360) = 294.6$) with a significant interaction ($p < 0.0001$; $F(45,360) = 24.9$). Analysis of delta body weight also demonstrated a significant effect of group ($p < 0.0001$; $F(3,24) = 48.82$), time ($p < 0.0001$; $F(16,384) = 142.8$), and an interaction ($p < 0.0001$; $F(48,384) = 110.8$). Similarly, analysis of body weight showed a significant effect of group ($p < 0.0001$; $F(3,24) = 79.85$), time ($p < 0.0001$; $F(16,384) = 142.8$), and an interaction ($p < 0.0001$; $F(48,384) = 110.8$). Further Bonferonni post-hoc analysis showed on day 11, Old DIZE animals demonstrated a significant decrease in food intake compared to Old Control animals, whereas Young DIZE animals