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Recombinant human ciliary neurotrophic factor reduces weight partly by regulating nuclear respiratory factor 1 and mitochondrial transcription factor A

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Abstract

Ciliary neurotrophic factor (CNTF) can lead to weight loss by up-regulating energy metabolism and the expression of UCP-1 in mitochondria. To investigate the up-stream regulators of the expression of UCP-1, recombinant human CNTF (rhCNTF) (0.1, 0.3, 0.9 mg/kg/day s.c.) administered to KK-Ay mice for 30 days resulting in reductions in body weight and perirenal fat mass. In brown adipose tissues, the gene expressions of nuclear respiratory factor (NRF)-1, mitochondrial transcription factor A (TFam) and uncoupling protein (UCP)-1 were found up-regulated by rhCNTF. To the best of our knowledge, these effects represent new insights on the mechanisms of action of weight loss by rhCNTF. In addition, we also found that rhCNTF increased the activity of mitochondrial complex IV. The stimulation of NRF-1, TFam, UCP-1 and the enhanced activity of mitochondrial complex IV may be associated with remedying obesity. The result indicates that rhCNTF can enhance the expressions of NRF-1 and TFam, both of which can up-regulate the expression of UCP-1.

Keywords: Recombinant human ciliary neurotrophic factor; Obesity; Uncoupling protein-1; Nuclear respiratory factor-1; Mitochondrial transcription factor A

1. Introduction

The worldwide incidence of obesity has greatly increased. Obesity/abdominal obesity, type 2 diabetes, hypertension and dyslipidemia are closely linked together and clustering of these medical disorders in an individual has been labeled the "metabolic syndrome" (Unger, 2002). Body weight reduction is an important strategy for its treatment.

Ciliary neurotrophic factor (CNTF), a member of gp130 family, is an injury- and metabolic activity-induced up-regulation factor (Watt et al., 2006) and has now been identified as an anti-injury factor and anti-obesity agents (Kelleher et al., 2006; Zvonic et al., 2005). CNTF was found to possess anti-obesity activity now, but the mechanism of this action is unclear. When CNTF was administered to individuals suffering from amyotrophic lateral sclerosis (Guler et al., 2000) and to mice (Lambert et al., 2001), it caused anorexia and weight loss. Furthermore, CNTF-induced-weight loss appears to be mediated through leptin-like and leptin-independent mechanisms. Other studies have also shown that CNTF decreases body weight and food intake in various mouse models of obesity such as leptin deficient *ob*/*ob* mice, leptin-resistant *db*/*db* mice, diet-induced obese AKR/J mice and UCP-1 diphtheria toxin A mice (Sleeman et al., 2003; Bluher et al., 2004). The way in which CNTF affects the gene expression of nuclear or mitochondrial uncoupling protein-1 (UCP-1) has not been reported.

The transcriptional control of mitochondrial biogenesis requires the expression of a large number of genes encoded by the nuclear and mitochondrial genetic systems (Andersson and Scarpulla,

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2001; Franco et al., 2006). Nuclear genes play a predominant role in controlling mitochondrial transcription, translation, and DNA replication (Natalie et al., 2005). The coding genes of the regulating factors for transcription, duplication and the processing of mitochondrial DNA (mtDNA) exist in nuclear DNA (nDNA) (Bergeron et al., 2001). It has been suggested that the transcription factor, nuclear respiratory factor-1 (NRF-1) plays a role in the cellular adaptation to energy metabolism by transducing a metabolic perturbation into an increased capacity to generate energy by transcription factor binding the DNA binding region.

Cytochrome *c* is present in mitochondria and is an essential component of the electron transfer chain involved in the transfer of electrons between complex III and complex IV. It is a marker for content of mitochondria. The activity of complex IV is a marker of mitochondrial respiration. We were also interested in whether the membrane potential of mitochondria changed, because the increased UCP-1 in mitochondrion may lead to low membrane potential.

The aim of the present study was investigating the anti-obesity mechanism of action of recombinant human CNTF (rhCNTF). We investigated the expression of NRF-1, mitochondrial transcription factor A (TFam) and UCP-1 and also determined the content of cytochrome *c*, the activity of complex IV and membrane potential of mitochondria in brown adipose tissue obtained from obese KK-Ay mice. These mice are obese because the central leptin signaling cascade is disrupted causing alimentary-induced obesity and insulin resistance. It is enough for the use in this study because we were focusing upon the effects of CNTF on the regulation of UCP-1.

2. Methods

2.1. Reagents

RhCNTF (molecular mass 21,244 Da) was developed by China C-bons pharmaceutical corporate (Hubei China). It comprised 186 amino acids in the following sequence:MAFTEHSPLTPHRRDLCSRSIWLARKIRSDLTALTESYVKHQGLNKNINLDSADGMPVASTDQWSELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAIHTLLLQVAAFAYQIEELMLLEYKIPRNEADGMPINVGDGGLFEKKLWGLKVLQELSQWTVRSIHDLRFISSHQTG.

TRIzol® and Superscript™ III reverse transcriptase were obtained from Invitrogen Corporation (Life Tech Co., Ltd. TakaRa Taq ™DNA polymerase (TakaRa Biotechnology Co. Ltd (Dalian China), ribonuclease inhibitor and Random Primer were purchased from TakaRa Biotechnology Co., Ltd (Dalian China). Triiodothyronine (3,3′,5′-triiodo-L-thyronine (T3); 98% purity) was purchased from Sigma Chemical Co., Ltd (USA). All other chemicals were of analytical grade and purchased/obtained from Chinese Beijing Xinjingke corporate.

2.2. Animals

The experiments were carried out in compliance with the guidelines for animal care and use of China and the experimental protocols were approved by the animal ethics committee of the Chinese Academy of Medical Sciences and Peking Union Medical College. Fifty-five genetically obese KK-Ay mice (30±3 g, aged 6 weeks, male or female randomly) were single-housed for 30 days in a light (12 h on and 12 h off), temperature (23±2 °C) and humidity (40–60%) controlled specific-pathogen free environment, with a high caloric (4.61 kcal/g) mice chow and water available *ad libitum*. The mice were obtained from and the diet was prepared by the Institute of Laboratory Animal, Chinese Academy of Medical Sciences and Peking Union Medical College.

The mice were divided equally into five groups: a control group (Veh) in which normal saline was injected subcutaneously for 30 days; a positive control group (Con) in which 0.4 mg/kg T3 was injected subcutaneously for 30 days (Mracek et al., 2005); a low dose treatment group (CL) in which 0.1 mg/kg rhCNTF was injected subcutaneously for 30 days; a medium dose treatment group (CM) in which 0.3 mg/kg rhCNTF was injected subcutaneously for 30 days; and a high dose treatment group (CH) in which 0.9 mg/kg rhCNTF was injected subcutaneously for 30 days. Food intakes and body weights were measured every 48 h. Mice were acclimated to single-housing and daily human handling to reduce stress for at least one week prior to experimental procedures (Zhang et al., 2006).

To investigate the short term effects of rhCNTF, five mice in each group were removed from the experiment after three days of treatment, i.e. on the fourth day. These animals were killed by decapitation and the brown adipose tissues between the scapulae were surgically removed and used for preparing mitochondria and the various assays (see later for details). At the end of the 30 days treatment, the remaining mice in each group were killed by decapitation and the body weights and the perirenal fat mass were measured/determined.

2.3. Detection of nuclear and mitochondrial NRF-1, TFam, UCP-1 gene transcripts by RT-PCR

Total RNAs were extracted from brown adipose tissues removed from mice treated for three days with TRIzol® and centrifuged at 12,000 g for 10 min at 4 °C. The RNA pellets were stored at −40 °C until use. For conducting semi-quantitative RT-PCR to assess the expression of 1 μg total RNA and first-strand cDNA synthesis, the pellets were re-suspended in sterile ribonuclease-free water and incubated at 95 °C for 4 min. RT-PCR was performed according to the directions included with the SuperScript™ system in a 25 μl reaction volume using the Techne Genius PCR equipment (Made by Techne Ltd. DUXFORD Cambridge England), PCR amplifications employed 29 cycles with steps at 95 °C for 40 s, 52 °C for 45 s, and 72 °C for 45 s followed by elongation at 72 °C for 7 min. The presence of specific PCR products was confirmed by visualization on a 1.5% agarose gel stained with ethidium bromide, and analyzed by the Kodak digital imaging system (Kodak DC120, Digital Science 1 D System, USA). The results are expressed as ratios relative to β-actin (density of PCR product/density of β-actin). The forward and reverse sequences used for RT-PCR are listed in Table 1. They are all identified in GeneBank.