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Introduction

One of the most prevalent health issues that continue to affect a growing number of Americans in the twenty-first century is obesity. In just the past 50 years, the number of overweight and obese individuals has risen to 64% of adults and 25 million children in the U.S. (Ogden *et al*., 2008). Obesity not only causes lifestyle limitations but other health problems as well, including coronary heart disease, type II diabetes, cancer, hypertension, dyslipidemia, stroke, liver disease, gallbladder disease, sleep apnea, respiratory problems, osteoarthritis, and reproductive problems in women (Pi-Sunyer, 2002; Ogden *et al*., 2008). Therefore, the exploration of different means to combat obesity has been brought to the forefront of medicine over the past decade.

A number of studies have looked at treating leptin deficiency, one of the main causes of obesity, through gene therapy with recombinant adenoviruses, direct administration of leptin either intracerebroventricularly or peripherally, and using skin explants from transgenic mice over-expressing leptin (Chen *et al*., 1996; Christensen *et al*.,1999; Muzzin *et al*., 1996; Larcher *et al*., 2001). Unfortunately, each of these approaches has a number of limitations, rendering the treatment either ineffective or unable to produce long-lasting results. In 2001, however, it was found, while examining the effects of ciliary neurotrophic factor in humans to treat motor neuron disease, that there was a substantial weight loss in test subjects over the course of treatment (Lambert *et al*., 2001). The effectiveness of CNTF in weight loss can be attributed to its binding to a different receptor that activates a similar JAK-STAT signaling pathway to moderate food intake and perception of hunger, just as leptin does (Gloaguen *et al*., 1997; Kelly *et al*., 2004; Lambert *et al*., 2001).

An additional advantage of CNTF in treating obesity is that it appears not only to be effective in *ob/ob* mice, where leptin hormone protein is nonfunctional, but also in *db/db* mice, which are lacking a functional leptin receptor (Gloaguen *et al*., 1997; Kelly *et al*., 2004; Lambert *et al*., 2001). This means CNTF could actually lead to a universal treatment for obesity. It was also noted by Lambert *et al.* (2001) that unlike dieting and other treatments for obesity, treatment with CNTF resets the "hypothalamic weight setpoint" – after stopping the administration of CNTF there is no immediate rebound weight gain due to increased appetite. Given the initial potential CNTF treatment shows for reversing the obesity phenotype, it seems pertinent to look at several different aspects of CNTF administration and its effect on gene expression.

Aim of the Study

To determine the effects of CNTF administration and detect related changes in gene expression, the following will be examined:

1. The maximum effective dose of CNTF, where no visible adverse effects are apparent and the longevity of weight loss effects is maximized between additional CNTF treatments.
2. The potential transcriptional up-regulation of genes in various brain regions related to increased levels of CNTF.

Experimental Proposal

Maximum Effective Dose of CNFT in ob/ob Mice

In the development of drug therapies, it is necessary to balance both the associated positive and negative effects. While CNTF appears to be successful in reducing body weight, it has also been shown that high levels of CNTF can induce fever and inflammatory response pathways (Kelly *et al*., 2004). Therefore, the first goal of this study is to examine various doses of CNTF to determine which µg/kg ratio is most effective in reducing body weight and not inducing any apparent adverse effects. Additionally, after stopping administration of CNTF, mice will be monitored to determine whether they start to regain weight depending on the particular dosage.

To look at these relationships, knockout mice for the *Ob* gene will be generated as described by Larcher *et al.* (2001) using transfected embryonic stem cells inserted into mouse embryos and the NEO^R-tk system to delete the *Ob* gene. The resulting *ob/ob* mice, which will express the obesity phenotype due to leptin deficiency, will be injected intravenously with different doses of recombinant human CNTF or a saline solution (Kelly *et al.,* 2004). A previous study by Kelly *et al.* (2004) demonstrated that CNTF, unlike leptin, is able to penetrate the blood-brain barrier, making peripheral injections a plausible treatment option.

Recombinant human CNTF will be produced by isolating the *Ob* gene using PCR amplification, cloning the gene product into the bacterial expression plasmid pRSET-5d that can incorporate larger gene fragments, and inserting the plasmid into *E. coli* cells (Gloaguen *et al*., 1997). Given that CNTF is usually expressed at 100-150 µg/kg in mice, injections >150 µg/kg would represent levels that are greater than what is normally expressed in mice. Therefore, the following amounts of CNTF will be administered to experimental groups of mice (n=6): 100µg/kg, 150µg/kg, 200µg/kg, 250µg/kg, 300µg/kg, 350µg/kg, and 400µg/kg. This range allows for the comparison of normal levels of CNFT with elevated levels of CNFT. The control group of mice (n=4) will be injected in the same fashion with 250 µg/kg of a saline solution. All mice will be injected once a day for 5 weeks. During this time, the weight and body mass index of each mouse will be monitored.

After 5 weeks, the mice will no longer be injected with either recombinant human CNTF or the saline solution. For an additional 5 weeks, the weight and body mass index of each mouse will be monitored to observe whether any mice begin to regain weight in relation to the dosage of CNTF.

Based on limited knowledge from prior studies, two observational trends are expected: 1) as the amount of CNFT increases, the rate at which weight loss occurs will increase to a certain extent (at some point this effect will plateau because all CNFT receptors will be occupied), and 2) as the amount of CNFT increases, there is a greater likelihood that fever and inflammation will occur. In addition, at higher doses of CNFT, the weight loss effects will persist over a longer period of time, reducing the need for frequent CNTF administration.

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If no adverse effects are seen between different experimental groups in relation to the control group this could mean that either the dosage of CNTF is too low to produce negative effects or these effects are not manifesting themselves physically. This would support studying the effect of increased CNTF level on the genomic level.

Examination of the Up Regulation of Genes due to Increased Levels of CNFT

CNTF has a number of different functions in the nervous system, with instigation of weight regulation and inflammatory response pathways being just two of these different effects. Aside from these functions, this protein hormone also promotes neurotransmitter synthesis, neurite outgrowth in astrocysts, and it initiates other transcriptional pathways (Gloaguen *et al*., 1997; Lambert *et al*., 2001). Therefore, by altering the level of CNTF in the body, a number of other pathways could be influenced in the process, upsetting the balance of actively transcribed proteins and other gene products. In relation to this prediction, it would be necessary to examine the change in gene expression due an increase in CNTF and identify the gene products to determine whether alteration in gene expression would be deleterious.

To look at the effects of CNTF at the genomic level, an experimental group of mice (n=6) will receive daily intravenous injections of 300 µg/kg of recombinant human CNTF for 3 weeks. This dose of CNTF will be used to represent significantly elevated levels of CNFT, while avoiding a severe inflammatory response (Kelly *et al*., 2004). Additionally, a control group of mice (n=6) will receive 300 µg/kg of a saline solution over the same duration.

Once a week, over the course of 3 weeks, functional magnetic resonance imaging will be completed on all mice in the experimental group approximately 30 minutes after injection with recombinant human CNTF (Farooqi *et al*., 2007). Results from fMRI will allow for identification of regions of the brain that are activated in response to CNFT. After 3 weeks, both experimental and control mice will be sacrificed in compliance with the Institutional Animal Care and Use Committee. Based on the brain regions that are activated by CNFT, tissue samples will be collected from these corresponding regions in both CNTF and saline-injected mice. The CNFT and saline mice brain cells will be cultured separately *in vitro* following the methods set forth by Gloaguen *et al*. (1997). RNA will be obtained from each cell line to generate representative cDNA for the genes expressed in each cell type. The cDNA from CNTF-injected mice will be fluorescently labeled with Cy3 (green) dye, and cDNA from saline-injected mice will be labeled with Cy5 (red) dye. Microarray analysis will then be completed to compare the difference in gene expression between the CNTF-injected and saline-injected mice to determine influence of increased levels of CNTF (Cell Press, 2005). Using this technique, the genes influenced by CNTF can be directly identified, and it will be possible to determine whether their altered activity can have negative consequences. Further studies would be necessary to examine the effects of these genes specifically.

It is expected that there will be a number of genes that are either up or down-regulated in the presence of higher levels of CNTF, especially in the hypothalamic region of the brain (Farooqi *et al*., 2007). While this approach can help to elucidate associated deleterious effects with gene expression, it does directly examine the activation of signaling pathways. This approach presents only one way in which the effects of CNTF can be measured, and it would be necessary to examine other aspects of CNTF's role in the brain.

Conclusion

The use of ciliary neurotrophic factor as a potential treatment for obesity appears promising because it provides an alternative pathway in which weight can be moderated. However, the alteration of hormone levels often will not only affect one desired pathway but also other processes in which it is involved. Results from this study will provide further insight into how the dosage of CNTF influences weight loss and instigates adverse effects over time, as well as how increased levels of CNTF influence the other transcriptional pathways. In order to further develop CNTF administration as a possible treatment for obesity, a number of other factors need to be assessed, especially for long-term treatment with CNTF.

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