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Increased HDAC in association with decreased plasma cortisol in older adults with chronic fatigue syndrome

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ABSTRACT

Hypocortisolism is a frequent finding in individuals with chronic fatigue syndrome (CFS) with other research findings implying potential dysregulation of glucocorticoid signaling.

Glucocorticoid signaling is under the influence of several pathways, several of which are of interest in the study of CFS. Oxidative stress and decreased antioxidant capacity are known to disrupt the hypothalamic–pituitary–adrenal (HPA) axis (Epel et al., 2004) and the presence of histone deacetylases (HDAC) could also impact glucocorticoid signaling. The intent of this pilot study was to investigate the relationship among oxidative stress elements, select HDAC's (2/3) and glucocorticoid receptor signaling in an elderly sample with CFS. Findings suggest increased histone deacetylase activity, lower total antioxidant power, in the context of decreased plasma cortisol and increased plasma dehydroepiandrosterone concomitant with decreased expression of the encoding gene for the glucocorticoid receptor. These findings support the presence of HPA axis dysregulation in elderly individuals with CFS.

1. Introduction

In adult populations, dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis is associated with chronic fatigue syndrome (CFS), suggesting an altered physiological response to stress (Johnson and DeLuca, 2005). Several studies have implicated glucocorticoid regulation with CFS, with a range of findings demonstrating that adults with CFS display lower levels of cortisol (Cleare, 2003; Gaab et al., 2002), a possible lack of responsiveness on the part of the HPA axis to challenge (Dinan et al., 1997), a pattern of glucocorticoid resistance (Kavelaars et al., 2000), and disruption or dysregulation of the expected diurnal cortisol pattern among patients with CFS (Torres-Harding et al., 2008). Previously, we found decreased genetic expression of the glucocorticoid receptor (NR3C1) concomitant with low levels of plasma cortisol in those with CFS (Jason et al., 2010). The presence of decreased message in the face of decreased hormone led to a conceptualization of epigenetic dysregulation of GR signaling. Based on the literature, we concluded that HDAC's were the most likely culprit. This pilot study was designed to test that belief.

Histone deacetylases (HDAC) are a group of enzymes with pronounced effects on gene regulation. These enzymes inhibit gene regulation through removal of acetyl groups, which ultimately decreases gene transcription (Yuan et al., 2009). This mechanism of action is the primary means through which glucocorticoids exert effects. HDAC's appear to be recruited by glucocorticoids, and in turn suppress the encoding of inflammatory genes contributing to the overall anti-inflammatory action of glucocorticoid (Adenuga and Rahman, 2007). An increase in HDAC activity, in the presence of decreased glucocorticoid concentrations may reflect a dysregulation of pathway signaling that could contribute to a chronic pro-inflammatory state. While HDAC activity may suppress inflammation through effects on the NFκB pathway, elements of oxidative stress may still be able to initiate inflammation through effects on the activator protein-1 pathway (Rahman, 2003). Differential pathway activation may explain the divergent immunologic findings observed in those with CFS. Oxidative stress, a state of chronic inflammation, may decrease expression of HDAC providing a mechanism through which inflammatory processes can influence the expression of HDAC, thus influencing the expression of other genes and influencing cell proliferation. It is also possible that the activity of HDAC's are reduced in certain disease states (Barnes, 2006). It is then important to investigate the relationship between oxidative stress and HDAC in those with CFS.

A close link between decreased glucocorticoid sensitivity and oxidative stress has been extensively described (Adcock et al.,

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2005), that could be applicable to CFS. Oxidative stress is linked with glucocorticoid resistance by affecting several aspects of GR activation and function including reduced GR nuclear transport (Okamoto et al., 1999), reduced GR transcription via decreases in histone deacetylase (HDAC) activity (Adcock et al., 2005) and decreased expression of glucocorticoid regulatory genes.

The primary intent of this pilot study was to determine the baseline characteristic of gene expression as it affects a discrete component of the HPA axis in a geriatric population with CFS. Specific hypotheses included that individuals with CFS would exhibit: (1) decreased expression of NR3C1 concomitant with decreased levels of plasma cortisol; (2) increased expression and activity on the part of class I HDAC's. Plasma DHEA and total antioxidant power were evaluated in order to evaluate other variables that may influence cortisol secretion in this population.

2. Methods

2.1. Sample

Data were obtained on nine individuals who were 65 years of age or older and diagnosed with CFS using the Fukuda criteria (Fukuda et al., 1994). The sample included 6 females and 3 males. The average age was 69 (range 65–79). Serum cortisol served as an indicator of HPA axis function. Samples were obtained from Judy Mikovitsof the Whittemore Peterson Institute for Neuroimmune Disease.

2.2. Cortisol and DHEA

Cortisol concentrations were evaluated through the use of a commercially available enzyme linked immunoabsorbent assay (sensitivity: .030–3111 ng/ml) obtained from R&D systems (R&D Systems, Minneapolis, MN). As cortisol itself is influenced by another hormone, dehydroepiandrosterone (DHEA), which has been implicated in the pathogenic process of fatigue (Cleare, 2003) it was felt important to examine for the presence of this hormone as well. A commercially available enzyme immunoassay kit (EIA) was used to determine the concentration of DHEA in serum. This array has been found to have a sensitivity of 2.90 pg/ml with a recovery of 98.5% (Assay Designs, Ann Arbor, MI).

2.3. Oxidative stress and antioxidant measures

It is important to determine an organism's homeostatic ability to counter the effects of oxidative stress. This potential is often decreased in the presence of disease and can be measured through performance of an array that measures the ferric reducing ability of plasma (FRAP).

The redox status of peripheral blood mononuclear cells (PBMC) was assessed as these cells are exposed during inflammatory processes to ROS, which requires an effective GSH capacity to neutralize ROS that could otherwise disrupt immune functions (Nakamura et al., 1997). Isolated PBMC was disrupted by ultrasonication in two 10-s sessions. The cytosolic fraction was purified by ultracentrifugation at 100,000*g* for 1 h at 4 °C. HDAC activity was measured on PBMC cells using the HDAC colorimetric activity assay kit from Active Motif (Carlsbad, CA) according to their assay protocol.

A decrease in the production of HDAC may in turn contribute to a chronic pro-inflammatory state, which may result in the expression of fatigue. PBMC nuclear cell extracts were incubated with HDAC assay substrate for 60 min. The assay substrate is a short peptide containing an acetylated lysine residue, which can be deacetylated by HDAC enzymes. After the 60 min incubation, developing solution was applied which reacts with the deacetylated lysine residue forming a yellow color which absorb maximally at 405 nm. The absorbance of the samples was read at 405 nm using a Victor Wallace plate reader (Perkin Elmer, MA). The absorbance values are directly proportional to the amount of deacetylated product formed representing a measure of HDAC activity.

The FRAP measure of total antioxidant power was measured by the method of Benzie and Strain (1996). The FRAP assay assesses "total antioxidant power" using antioxidants as reductants in a redox-linked colorimetric method and describes the status of plasma antioxidants apart from protein sulfhydryls. The components of plasma that contributed to total antioxidant capacity in percentage terms are estimated as ascorbate 15%, alpha-tocopherol 5%, uric acid 60%, bilirubin 5% and remaining antioxidants 15%. The Fe^{3+}–TPTZ (ferric tripyridyltriazine) complex is reduced at a low pH to the blue ferrous form, Fe^{2+}. $FeSO_4{\cdot}7H_2O$ and L-ascorbic acid are used as standards. The absorbance at 593 nm is taken at 6 and 12 min after incubation at room temperature using an Automated Microplate Reader (KC4, Bio-Tek Instruments, Inc., Winooski, Vermont, 2000). The change of absorbance is linearly proportional to the concentration of antioxidant.

2.4. Gene expression

Pelleted cells were sent to Panomic's Inc. (Fremont, CA) for examination of mRNA transcripts. The assay service was blind to the clinical population from which cells were obtained, and used a Quantigene Plex system to examine for previously selected transcripts. This system employs cell lysate and branched DNA in creating a sandwich nucleic acid hybridization, which is then bound to a biotinalyted probe. Results are obtained through the use of laser excited fluorescent signal. Sample mean fluorescent intensity was calculated in relation to the mean signal of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Sample plates were read through the use of a BioPlex System (BioRad Laboratories, Hercules, CA).

Rather than investigate polymorphisms in the GR, we examined for the presence of RNA transcripts affecting receptor expression. One transcript involved in receptor acetylation was NR3C1 (nuclear receptor subfamily 3, group C, member 1 GR). By investigating this factor, we examined for variance in the expression of a factor involved in the regulation and expression of a discrete element of the HPA axis in individuals with CFS. Our rationale for the selection of this gene was as follows: polymorphisms of the NR3C1 gene have been associated with CFS in adults (Rajeevan et al., 2007), and this gene is one of the main transcriptional regulators of the GR. Additional transcripts examined for included HDAC2, HDAC3, SIRT1, and NGF.

Sirtulin-1 (SIRT1) is a major regulator of the p53 pathway and appears to decrease apoptosis in the presence of stress. SIRT1 also appears to influence circadian gene expression (Belden and Dunlap, 2008). The p53 pathway is also influenced by histone deacetylases which are associated with corticosteroid dysregulation. Previously, we found a decrease in mRNA transcripts for NR3C1, concomitant with lower levels of cortisol in a population of adults with chronic fatigue (unpublished data). Decreased message for the glucocorticoid receptor in the presence of decreased hormone suggested dysregulation of signaling. The most likely gene candidates based on previous data were HDAC2 and HDAC3. These were chosen due to their influence on the NFκB pathway and interrelationship with glucocorticoid (Barnes, 2006; Ito et al., 2006). We also chose to examine for the presence of nerve growth factor (NGF) which has been found associated with pain conditions and implicated in CFS (Seidel et al., 2010).

2.5. Statistical procedures

All variables were treated on a continuous scale in statistical analyses. Variables that failed the normality test were logarithmically transformed before the analysis to allow for assessment