Interferon-alpha-induced inflammation is associated with reduced glucocorticoid negative feedback sensitivity and depression in patients with hepatitis C virus

Jennifer C. Felger,⁎ Ebrahim Haroon, Bobbi J. Woolwine, Charles L. Raison, Andrew H. Miller

Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA 30322, United States
The Winship Cancer Institute, Emory University, Atlanta, GA 30322, United States
School of Human Ecology, University of Wisconsin-Madison, Madison, WI 53706, United States
Department of Psychiatry, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53706, United States

HIGHLIGHTS

• IFN-alpha decreased glucocorticoid sensitivity in association with flattened diurnal cortisol slope in patients with hepatitis C virus
• Decreased glucocorticoid sensitivity was correlated with increased IFN-alpha-induced soluble tumor necrosis factor receptor 2 (sTNFR2)
• Increased sTNFR2 predicted increased depression and fatigue scores, independent of the change in glucocorticoid sensitivity
• Inflammation in medical illness may decrease glucocorticoid sensitivity, in turn increasing cytokines and their effects on behavior

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ABSTRACT

Major medical illnesses are associated with increased risk for depression and alterations in hypothalamic–pituitary–adrenal (HPA) axis function. Pathophysiological processes such as inflammation that occur as a part of medical illnesses and their treatments have been shown to cause depressive symptoms, and may also affect the HPA axis. We previously reported that patients with hepatitis C virus chronically administered interferon (IFN)-alpha develop increased evening plasma cortisol concentrations and a flattened diurnal cortisol slope, which correlated with increased tumor necrosis factor (TNF) and its soluble receptor 2 (sTNFR2). Increased TNF and sTNFR2 were further correlated with depression and fatigue scores. The current study examined whether flattened cortisol slope might be secondary to reduced glucocorticoid receptor (GR) sensitivity, by measuring glucocorticoid negative feedback to dexamethasone (DEX) administration followed by corticotropin releasing hormone (CRH) challenge. In an exploratory analysis, 28 male and female patients with hepatitis C virus were studied at baseline (Visit 1) and after 12 weeks (Visit 2) of either IFN-alpha plus ribavirin (n = 17) or no treatment (n = 11). Patients underwent dexamethasone DEX–CRH challenge, neuropsychiatric assessments, and measurement of plasma TNF and sTNFR2 during each visit. IFN-alpha did not affect neuroendocrine responses following CRH but did increase post-DEX cortisol, which was correlated with flattening of the diurnal cortisol slope (r = 0.57, p = 0.002) and with increased depression scores (r = 0.38, p = 0.047). Furthermore, the change in post-DEX cortisol was associated with IFN-alpha-induced increase in sTNFR2 (r = 0.51, p = 0.006), which was in turn correlated with depression (r = 0.63, p < 0.001) and fatigue (r = 0.51, p = 0.005) scores. Whereas the relationship between sTNFR2 and depression scores were independent of the change in post-DEX cortisol, the correlation between post-DEX cortisol and depression scores was not significant when controlling for sTNFR2. These findings suggest that inflammation induced in patients with hepatitis C virus during IFN-alpha therapy precipitates decreased GR sensitivity to lead to a flattened diurnal cortisol slope. Decreased GR sensitivity may in turn further increase inflammation and its ultimate effects on behavior. Treatments that target inflammation and/or GR sensitivity may reduce depressive symptoms associated with medical illnesses.

⁎ Corresponding author at: Emory University School of Medicine, 1365-B Clifton Road, 5th Floor, Atlanta, GA 30322, United States.
E-mail address: jfelger@gmail.com (J.C. Felger).

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1. Introduction

Medical illnesses such as chronic viral infections, cancer and cardiovascular disease are associated with increased risk for depression as well as with changes in hypothalamic–pituitary–adrenal (HPA) axis function, including reduced glucocorticoid receptor (GR) sensitivity [1,12,36,53]. One pathophysiologic process that may account for relationships among medical illness, depression and HPA dysregulation is inflammation [20,55]. Increased inflammation has been observed in both medically ill and medically healthy patients with depression, and administration of inflammatory stimuli and cytokines to humans and laboratory animals causes depressive behaviors [10,23,35,46,65]. Indeed, administration of the inflammatory cytokine interferon (IFN)-alpha for malignant melanoma or hepatitis C virus (HCV) leads to a range of depressive symptom including anhedonia, anxiety, sleep disturbances and fatigue [17,48], all of which have been found to correlate with induction of other cytokines and inflammatory signaling pathways [13,14,42,50]. Similar relationships between inflammatory markers and depressive symptoms have been observed in other medical illnesses, particularly in cancer patients undergoing treatment with chemotherapy and radiation that, like IFN-alpha, activate endogenous production of inflammatory cytokines and gene expression pathways [3,5,61].

Medical illnesses such as cancer and the administration of IFN-alpha have also been associated with changes in HPA axis function [1,12,36,51,53,59]. For example, women with breast cancer have been found to exhibit higher blood cortisol concentrations following oral dexamethasone administration, which correlated with a more flattened cortisol slope [58]. Moreover, administration of IFN-alpha caused flattened cortisol slope and increased evening cortisol concentrations in patients with hepatitis C virus, both of which correlated with depression and fatigue scores. Depression and fatigue scores were in turn positively correlated with induction of the inflammatory cytokine tumor necrosis factor (TNF) and its soluble receptor 2 (sTNFR2) [45]. A number of studies have indicated that a flattened cortisol slope is associated with increased morbidity and mortality in the context of a variety of illnesses including diabetes, cardiovascular disease and cancer [1,12,51,52,59]). The mechanism of this altered diurnal cortisol rhythm in inflammation-related illness is unknown, although data suggests that changes in GR sensitivity may be involved [37]. In the current study, we examined whether the flattened cortisol slope in patients with hepatitis C virus administered IFN-alpha may occur secondary to alterations in glucocorticoid negative feedback sensitivity as measured by the dexamethasone (DEX) suppression test followed by administration of corticotropin releasing hormone (CRH) (the DEX–CRH challenge) [21,22], and whether changes in sensitivity to DEX were associated with previously described IFN-alpha-induced increases in plasma TNF and sTNFR2 and depressive behaviors [45].

2. Methods and materials

2.1. Participants

Twenty-eight HCV-positive subjects (14 males, 14 females) were enrolled. Exclusion criteria included decompensated liver disease; liver disease from any cause other than HCV; unstable cardiovascular, endocrinologic, hematologic, renal or neurologic disease (as determined by physical exam and laboratory testing); infection with HIV (as reported by the subjects’ treating physician); and history of schizophrenia or bipolar disorder and/or a diagnosis of major depression or substance abuse/dependence within 6 months of study entry, as determined by Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders- Fourth Edition [18]. Patients were required to be off all antidepressant, antipsychotic, or mood stabilizer medications for at least 4 weeks prior to study entry (8 weeks for fluoxetine). Subjects were also required to discontinue other agents that might affect study results (i.e., narcotic analgesics, benzodiazepines, and anti-inflammatory agents) at least 2 weeks prior to sample collection. The subjects reported on herein represent a subsample of subjects included in previous studies on the effects of IFN-alpha on depressive behaviors, cognitive performance, neuroendocrine function, gene expression, and inflammatory responses [13,16,43,45,47,45]. All subjects provided written informed consent, and study procedures were approved by the Emory University Institutional Review Board.

2.2. Study design

Study participants were enrolled in a longitudinal study examining immune, neuroendocrine, and neuropsychiatric variables at baseline and after either no treatment or chronic treatment with IFN-alpha/ribavirin [13,14,16,44,45,47]. For purposes of this study, DEX–CRH challenge involved administration of DEX at 2300 h followed by collection of 6 post-DEX cortisol and adrenocorticotropic hormone (ACTH) samples from 1230 to 1300 h the following day (DEX suppression test) prior to administration of CRH and collection of 6 subsequent samples over 2 h (CRH challenge) as previously described [21,22]. The DEX–CRH challenge was performed at baseline (Visit 1) and at 12 weeks (Visit 2) from a subset of HCV+ patients treated with IFN-alpha plus ribavirin (n = 17) or untreated HCV+ patients awaiting IFN-alpha/ribavirin therapy (control subjects, n = 11). All subjects who underwent IFN-alpha treatment received either pegylated IFN-alpha-2b (Peginteron, Schering Plough, Kenilworth, NJ; 1.5 μg/kg)(n = 9) or pegylated IFN-alpha-2a (Pegasys, Roche-Genentech, San Francisco, CA; 180 mg)(n = 8) weekly administered subcutaneously plus oral ribavirin (800–1400 mg) daily. Participation in the treatment versus control group was determined by the patients and their physicians based on scheduling constraints and personal preferences and was not based on standardized criteria or controlled by study protocol.

For DEX–CRH challenge at Visits 1 and 2, subjects were admitted to the Emory University Clinical Research Network (CRN) for evaluation. To allow for accommodation to the CRN environment and to control for the effects of sleep and waking times on diurnal cortisol/ACTH and cytokine rhythms, subjects were admitted to the CRN at least 12 h prior to commencing blood draws for evaluation of hormones, TNF and sTNFR2. Lights out occurred at 2200 h, and all subjects were awakened at 0715 h. The following morning, subjects were awakened and served breakfast. An intravenous catheter was inserted at 0800 h and blood was collected every hour from 0900 to 2100 h to measure diurnal cortisol slope, and mean diurnal TNF and sTNFR2 concentrations (due to lack of significant diurnal change in this cytokine and its receptor) prior to conducting the DEX–CRH test during Visit 1 compared to Visit 2 as described [45]. During this time, neuropsychiatric assessments were also conducted. For the DEX–CRH challenge, a dose of 1.5 mg dexamethasone (Roxane Laboratories, Columbus, OH) was administered orally at 2300 h following collection of the 2100 h diurnal sample. On the following day, an intravenous catheter was inserted at 1200 h and 6 blood samples were collected every 30 min starting at 1230 h to determine mean post-DEX cortisol and ACTH concentrations as a measure of DEX suppression, immediately followed by post-CRH challenge samples. Immediately after the 1500 h sample, a bolus intravenous injection of 1 μg/kg ovine CRH (Ferring, Suffern, NY, USA) was administered. Blood samples were collected at 5, 15, 30, 60, 90, and 120 min after CRH injection to determine maximal post-CRH hormone responses and the area under the curve (AUC). Blood was collected into chilled EDTA-coated tubes and immediately centrifuged at 1000 × g for 10 min at 4 °C, and plasma was removed and frozen at −80 °C until assay. Subjects were asked to rest quietly during blood sampling. Urine drug screens were conducted at both study visits to rule out substance abuse. Control subjects participated in all study procedures in parallel with IFN-alpha/ribavirin-treated patients. The timeline of diurnal plasma sampling, behavioral assessments, DEX–CRH challenge administration and respective sampling intervals are detailed in Fig. 1.
2.3. Neuropsychiatric assessments

Neuropsychiatric assessments were conducted at baseline and 12 weeks of study participation. Depression was evaluated using the Montgomery–Asberg Depression Rating Scale (MADRS) [33]. The MADRS is a 10-item, clinician-administered scale that assesses the severity of depressive symptoms. Fatigue was assessed using the self-report, 20-item Multidimensional Fatigue Inventory (MFI) [56].

2.4. Sample analysis

Commercially available immunoradiometric assay and radioimmunoassay kits were used for the assessment of plasma ACTH (ALPCO Diagnostics, Salem, NH, USA and Nichols Institute Diagnostics, San Juan Capistrano, CA, USA when available) and cortisol (DiaSorin Stillwater, MN, USA) respectively [45]. Intra and inter-assay coefficients of variation respectively were 2.8 and 5.7% (ALPCO) or 4.5 and 6.3% (Nichols) for ACTH and 8.5 and 12.7% for cortisol. Due to our previous findings that TNF and sTNFR2 were the only plasma cytokines to increase during IFN-alpha administration, our studies focused on plasma concentrations of TNF and sTNFR2, which were measured by high-sensitivity and Quantikine enzyme-linked immunosorbent assay (ELISA) kits, respectively (R&D Systems, Minneapolis, MN, USA). Assays were performed according to the manufacturer’s specifications, and were run in duplicate. Inter- and intra-assay variability were reliably <12% for TNF and <10% for sTNFR2. All biological samples were analyzed by research staff blinded to the clinical status of study participants.

2.5. Statistical analysis

Differences between groups were assessed using t tests, Chi-squared Tests, or Fisher’s Exact Test for categorical variables. To determine the effect of treatment on hormone responses to the DEX–CRH challenge, the change in mean post-DEX plasma cortisol and ACTH, and change in maximum and AUC post-CHR plasma cortisol and ACTH from Visit 1 to Visit 2 were calculated as post minus pre (i.e. the delta; Visit 2 -Visit 1). Multivariate general linear models were used to examine the effects of treatment (IFN-alpha plus ribavirin versus control) on delta cortisol and ACTH measures following DEX and CRH while adjusting for age, sex, race, body mass index (BMI), and history of major depression (MD). Post-CHR ACTH data for one IFN-alpha-treated subject was excluded from multivariate analysis due to a significant Grub's test for statistical outliers (p < 0.05). To determine the relationship between DEX sensitivity and cortisol slope, we calculated the diurnal cortisol slope by natural log-transforming cortisol values and using the δ value of the regression of 13 samples collected every hour from 0900 to 2100 h on the day prior to DEX administration, as described previously [1,4,19,45,53]. The delta diurnal cortisol values (change in diurnal slope from Visit 1 to Visit 2) were used for statistical comparison with delta post-DEX cortisol. Because larger beta values (i.e. values closer to zero) reflect a flatter slope [1], positive delta slope values reflected flattening of the diurnal slope over time. To determine relationships between inflammation and DEX sensitivity, Pearson correlation coefficients were calculated to evaluate the association between the delta post-DEX mean cortisol and the delta for TNF and sTNFR2 (change in mean diurnal concentrations from Visit 1 to Visit 2) in all subjects. To further evaluate associations between inflammation and DEX suppression in patients administered IFN-alpha, delta post-DEX cortisol was compared between patients who exhibited “high” versus “low” delta sTNFR2 (≥ versus < the median of delta sTNFR2) the by T test. To determine relationships between inflammation or DEX sensitivity with behavior, Pearson correlation coefficients were calculated to evaluate the association between the delta post-DEX mean cortisol and the delta for sTNFR2 with depression and fatigue (as measured by MADRS and MFI scores, respectively) in all subjects. All significant linear relationships were assessed in linear models using backward (significance level stay = 0.05) and forward (significance level entry = 0.05) selection while controlling for age, sex, race, body mass index (BMI), and history of major depression (past MD). Partial correlation coefficients were calculated to establish independent relationships between TNF and sTNFR2 and change in hormone responses while controlling for change in behavior, and vice versa, using linear regression models that included covariates. Tests of significance were two-tailed with alpha <0.05, and all statistics were conducted using SPSS software (Chicago, IL, USA).

3. Results

3.1. Subject characteristics

As shown in Table 1, no significant differences between IFN-alpha/ribavirin-treated subjects and controls were observed for relevant clinical characteristics including age, race, sex, BMI and past history of substance abuse. However, there was a trend toward a significant difference between IFN-alpha-treated subjects and controls for history of MD (p = 0.082). Therefore, history of MD was included as a covariate in all linear regression and general linear models, along with age, sex, race and BMI. It should also be noted that there were no differences between IFN-alpha-treated and control subjects in baseline depression (MADRS) and fatigue (MFI) scores. However, IFN-alpha-treated subjects displayed significant increases in depression (MADRS score: t = -3.58, df = 26, p = 0.001) and fatigue (MFI score: t = -4.25, df = 26, p < 0.001) after 12 weeks of treatment compared to controls. Eight out of 17 patients administered IFN-alpha plus ribavirin (47%) exhibited MADRS scores ≥15, consistent with clinically significant depressive symptoms [13,14,41].

3.2. DEX-induced suppression of cortisol was significantly decreased in patients administered IFN-alpha compared to controls, and correlated with the diurnal cortisol slope

To evaluate the effect of IFN-alpha administration on plasma hormone responses to DEX–CRH challenge, the change in mean post-DEX concentrations of cortisol and ACTH from Visit 1 (baseline) to Visit 2 (week 12) of the study (deltas), and the delta maximal and AUC cortisol

![Fig. 1. Timeline of procedures during study Visit 1 (baseline) and study Visit 2 (12 weeks). Patients with hepatitis C virus administered IFN-alpha plus ribavirin therapy or controls patients were admitted to the inpatient research clinic for all procedures. Timeline denotes time of day for hourly diurnal blood sampling, behavioral assessments, DEX and CRH administration and respective post-DEX–CRH sampling intervals.](image-url)
and ACTH responses to CRH were compared in IFN-alpha-treated subjects (n = 17) and controls (n = 11) while controlling for age, sex, race, BMI and history of MD. The mean delta post-DEX cortisol, but not ACTH, was significantly higher in IFN-alpha-treated patients compared to controls (F[1,27] = 7.85, p = 0.011) (Table 2), indicating that post-DEX cortisol suppression was significantly reduced by 12 weeks of IFN-alpha/ribavirin administration compared to control. Data in Table 2 are presented as the mean (± SD) for Visit 1 and Visit 2 that were used to calculate the deltas, along with the deltas (± SD), which were statistically compared. No other significant effects of IFN-alpha/ribavirin administration on post-DEX suppression or post-CRH stimulation were observed (all p > 0.05).

Consistent with our previous findings [45], IFN-alpha-treated patients compared to controls exhibited significantly increased delta diurnal cortisol slope (more positive change in the δ values) prior to DEX administration when controlling for age, sex, race, BMI and history of MD (F[1,26] = 17.93, p < 0.001) (Table 2), indicating a flattened cortisol slope following IFN-alpha exposure (mean delta slope IFN-alpha: 0.02 ± 0.04, mean delta slope control: -0.01 ± 0.03). Interestingly, delta post-DEX cortisol was significantly positively correlated with delta diurnal cortisol slope (r = 0.57, df = 26, p = 0.002), and delta post-DEX cortisol remained the most significant predictor of delta cortisol slope in both backward and forward linear regression models including age, sex, race, BMI and history of MD (p < 0.01).

### 3.3. Increased plasma sTNFR2 was associated with decreased suppression of cortisol in IFN-alpha-treated patients and the sample as a whole

Relationships between post-DEX cortisol suppression and induction of TNF and sTNFR2 were examined by correlating cortisol in IFN-alpha-treated patients and the sample as a whole 3.3. Increased plasma sTNFR2 was associated with decreased suppression of cortisol in IFN-alpha-treated patients exhibited signi

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 11)</th>
<th>IFN-alpha (n = 17)</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, SD)</td>
<td>45.5 (7.3)</td>
<td>47.4 (6.1)</td>
<td>0.450</td>
</tr>
<tr>
<td>Sex (n, %) males</td>
<td>5 (45.5)</td>
<td>9 (52.9)</td>
<td>0.700</td>
</tr>
<tr>
<td>Race (n, %)</td>
<td>Caucasian 6 (54.5)</td>
<td>8 (47.1)</td>
<td>0.334</td>
</tr>
<tr>
<td>Black 5 (45.5)</td>
<td>6 (35.3)</td>
<td>0.812</td>
<td></td>
</tr>
<tr>
<td>Hispanic 0 (0)</td>
<td>2 (11.8)</td>
<td>0.278</td>
<td></td>
</tr>
<tr>
<td>Asian American 0 (0)</td>
<td>1 (5.9)</td>
<td>0.082</td>
<td></td>
</tr>
<tr>
<td>History of MD (n, %)</td>
<td>0 (0)</td>
<td>4 (23.5)</td>
<td>0.014</td>
</tr>
<tr>
<td>History of substance abuse (n, %)</td>
<td>8 (72.7)</td>
<td>18 (69.2)</td>
<td>0.800</td>
</tr>
<tr>
<td>BMI (mean, SD)</td>
<td>28.5 (5.7)</td>
<td>31.4 (4.4)</td>
<td>0.142</td>
</tr>
<tr>
<td>Baseline MADRS (mean, SD)</td>
<td>3.5 (5.1)</td>
<td>3.1 (4.0)</td>
<td>0.607</td>
</tr>
<tr>
<td>Baseline MFI (mean, SD)</td>
<td>39.7 (12.8)</td>
<td>40.4 (15.2)</td>
<td>0.903</td>
</tr>
<tr>
<td>12 week MADRS (mean, SD)</td>
<td>3.3 (3.9)</td>
<td>14.8 (10.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>12 week MFI (mean, SD)</td>
<td>35.3 (13.3)</td>
<td>65.5 (20.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI, body mass index; MADRS, Montgomery–Asberg Depression Rating Scale; MD, major depression; MFI, Multidimensional Fatigue Inventory.

*Significant relationships are indicated in bold.

IFN-alpha administration to HCV + patients led to significantly increased cortisol concentrations following DEX administration, suggesting that IFN-alpha exposure produced a decrease in GR sensitivity. Consistent with our previous findings [45], IFN-alpha administration was also associated with flattening of the diurnal cortisol slope, which correlated with increased post-DEX cortisol. Increases in post-DEX cortisol concentrations were further correlated with IFN-alpha-induced increases in the TNF soluble receptor, sTNFR2. Although both the increase in post-DEX cortisol and the induction of sTNFR2 correlated with increased depression scores, the relationship between post-DEX cortisol and increased depression was not significant when controlling for the increase in sTNFR2. Conversely, the relationship between increased sTNFR2 and increased depression occurred independently of effects on post-DEX cortisol, and vice versa. These findings support the idea that flattening of the diurnal cortisol slope in patients administered IFN-alpha may be due to inflammatory cytokine effects on GR sensitivity. Moreover, these findings are consistent with the idea that inflammation and the production of...
cortisol during medical illness may independently contribute both to depressive symptoms and HPA axis dysregulation.

Although IFN-alpha–treated patients did meet clinical criteria for DEX suppression of cortisol (post-DEX cortisol <5 μg/dl, equivalent 50 ng/ml or μg/l) [25], the mean post-DEX cortisol [63] increased by an average of 23.8%, with the patients that exhibited the most change showing an ~60% increase in post-DEX cortisol. Our findings of decreased post-DEX suppression of cortisol along with flattened cortisol slope in patients with hepatitis C virus administered IFN-alpha is similar to that which has been observed in patients with metastatic breast cancer [58], and in patients with major depression [26,39]. Decreased sensitivity to DEX administration and flattened slope are related manifestations of HPA axis dysfunction, which has been shown to contribute to metabolic disease, including obesity and insulin resistance [7,9,63]. Therefore, understanding mechanisms that contribute to decreased DEX sensitivity in patients with psychiatric and medical illness may be relevant to preventing further stress-related metabolic complications and co-morbidities.

One of the most interesting findings of the present study was that although a moderate correlation between IFN-alpha-induced changes in DEX sensitivity and depression existed, this relationship was no longer significant when controlling for induction of sTNFR2. However, relationships were observed between increase in sTNFR2 with both depressive behavior and change in DEX sensitivity, which were both statistically independent of each other. Therefore, it is likely that inflammatory processes such as TNF signaling that have been shown to contribute to the potent effects of IFN-alpha on behavior may also affect the HPA axis. Indeed, we have previously found that increased TNF and sTNFR2 (which increases in response to increased TNF activity and is stable in the blood) were associated with IFN-alpha-induced depression [45].

In terms of the mechanisms by which IFN–alpha may have led to decreases in DEX sensitivity, in vitro studies have demonstrated that IFN-alpha can decrease GR function through activation of signal transducer and activator of transcription 5 (STAT5), which was in turn found to bind to the GR in the nucleus and thereby prevent GR-mediated gene transcription [24,38]. Other IFN-alpha-induced cytokines (e.g. TNF) can also activate inflammatory signaling pathways such as nuclear factor kappa B (NF-κB) and mitogen activated protein kinases (MAPKs), which can additionally inhibit GR signaling and GR translocation from the cytoplasm to the nucleus [11,40,64]. Indeed NF-κB, a lymphoprotein molecule through which cytokines activate inflammation, is well known to inhibit GR function via nuclear protein–protein interactions [57]. Moreover, there appears to be a reciprocal relationship between signaling through NF-κB versus GR with repeated demonstration of increased activation of NF-κB–associated genes in conjunction with reduced activation of GR-regulated genes in cancer patients and their caregivers, as

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 11)</th>
<th>IFN-alpha (n = 17)</th>
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<tbody>
<tr>
<td></td>
<td>Visit 1</td>
<td>Visit 2</td>
</tr>
<tr>
<td>Post-DEX (mean, SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean cortisol (μg/dl)</td>
<td>1.27 (0.38)</td>
<td>1.32 (0.29)</td>
</tr>
<tr>
<td>Max ACTH (pg/ml)</td>
<td>6.47 (2.40)</td>
<td>6.60 (3.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-CRH (mean, SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max cortisol (μg/dl)</td>
<td>7.77 (5.39)</td>
<td>6.47 (4.56)</td>
</tr>
<tr>
<td>Max ACTH (pg/ml)</td>
<td>27.77 (11.04)</td>
<td>25.45 (9.88)</td>
</tr>
<tr>
<td>AUC cortisol</td>
<td>14.98 (15.63)</td>
<td>12.21 (11.82)</td>
</tr>
<tr>
<td>AUC ACTH</td>
<td>57.92 (38.78)</td>
<td>48.76 (30.98)</td>
</tr>
</tbody>
</table>

Diurnal (mean, SD)

|                      |         |         |       |         |         |       |
| Slope cortisol      | −0.08 (0.03) | −0.09 (0.03) | −0.01 (0.03)** | −0.10 (0.03) | −0.08 (0.03) | 0.023 (0.04) |

Post-dexamethasone (DEX) cortisol and adrenocorticotropic hormone (ACTH) concentrations were the mean of 6 samples collected every 30 min between 1230 to 1500 h following administration of DEX at 2100 h the evening before. Post-corticotropin releasing hormone (CRH) cortisol and ACTH were collected at 5, 15, 30, 60, 90 and 120 min after i.v. CRH administered immediately following the 1500 h blood draw. Maximum (Max) values reflect the highest hormone values post-CRH, and post-CRH area under the curve (AUC) was calculated from the 1500 h sample collected immediately prior to CRH administration. Slope was calculated as the β value of the regression of natural log transformed plasma cortisol concentrations measured from 0900 to 2100 h prior to DEX administration. Post-corticotropin releasing hormone (CRH) cortisol and ACTH were collected at 5, 15, 30, 60, 90 and 120 min after i.v. CRH administered immediately following the 1500 h blood draw. Maximum (Max) values reflect the highest hormone values post-CRH, and post-CRH area under the curve (AUC) was calculated from the 1500 h sample collected immediately prior to CRH administration. Slope was calculated as the β value of the regression of natural log transformed plasma cortisol concentrations measured from 0900 to 2100 h prior to DEX administration.
conclusively form the results obtained from this exploratory study.

These hypotheses are largely speculative and cannot be determined independently of the change in post-DEX cortisol, which may reflect IFN-alpha effects on GR sensitivity in the periphery. This is consistent with the finding that increase in peripheral sTNFR2 was associated with depressive behavior, which likely involves cytokine effects on brain neurotransmitters and neurocircuitry [17,30], independently of the change in post-DEX cortisol, which may reflect IFN-alpha effects on GR sensitivity in the periphery. However, these hypotheses are largely speculative and cannot be determined conclusively form the results obtained from this exploratory study.

Additionally, IFN-alpha administration and the associated increase in cytokines may exert a direct stimulatory effect on cortisol production [54]. Indeed, although the precise mechanisms are unknown, circulating and intra-adrenal cytokines have been shown to directly increase glucocorticoid release by both potentiating the effects of ACTH and by stimulating steroidogenesis [27,60].

Although this study provides interesting data suggesting that inflammation in medical illness may independently influence the HPA axis function and behavior, the sample size was small. Nevertheless, this exploratory analysis revealed moderate effect sizes ($r \approx 0.5–0.6$) for relationships between induction of sTNFR2 and change in DEX sensitivity and behavior in the sample as a whole ($n = 28$). However, we were underpowered to detect significant relationships specifically within the IFN-alpha-treated patients ($n = 17$). It is worth noting that hepatitis C virus infection may be associated with a low-grade inflammation that is not as potent as the effects of IFN-alpha administration, but that may have contributed to relationships between change in sTNFR2, DEX sensitivity and behavior over time, even in control subjects not receiving IFN-alpha. Another limitation of this study includes the fact that all of the IFN-alpha-treated patients were concomitantly treated with ribavirin, which may contribute to the depressive burden of IFN-based hepatitis treatment [2]. However, IFN-alpha monotherapy for malignant melanoma has been associated with profound induction of depression and fatigue [6,34,48], indicating that treatment effects on behavior in this study were likely attributable to specific effects of IFN-alpha. Additionally, the present study was not a randomized experiment in which patients were experimentally allocated to treatment and control groups. Therefore, a trend for the treatment group to have a higher rate of past MD was observed. To limit the potential effect of history of MD or other demographic and clinical variables, appropriate covariates were controlled for in statistical models.

5. Conclusions

Overall, this study provided evidence that chronic IFN-alpha administration may decrease glucocorticoid negative feedback sensitivity through direct effects on the GR, or through GR effects via activation of TNF-related inflammatory signaling pathways, which in turn lead to flattening of the diurnal cortisol slope. Furthermore, peripheral inflammatory activity was found to be related to increased depressive symptoms in these patients, independent of the change in DEX sensitivity. These results support the hypothesis that increased inflammation in medical illness may contribute to decreased glucocorticoid sensitivity which in turn may further increase inflammation and its ultimate effects on behavior and HPA axis function.

Conflict of interest statement

All authors declare that there are no conflicts of interest, and all financial disclosures are listed for each author: In the previous 12 months Charles L. Raison has served as an advisor for Otsuka-Lundbeck and Pamlab, on the speakers bureau for Sunovion, Pamlab and Merck and delivered non-branded promotional lectures for Otsuka; Jennifer C. Felger, Ebrahim Haroon, Bobbi J. Woolwine, and Andrew H. Miller have nothing to declare.

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References

[1] H.C. Akerboom, J. Giese-Davis, S. Septon, E.S. Epel, J.M. Turner-Cobb, D. Spiegel, Fat-


