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Citalopram Reduces Endotoxin-Induced Fatigue

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Abstract

Increased levels of inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6) may play a role in depression. Mild depressive-like symptoms can be induced in humans through activation of the innate immune system with endotoxin. Whether preventive treatment with antidepressants can reduce endotoxin-induced symptoms has never been tested. In a double-blind, randomized, placebo-controlled, cross-over study, we administered intravenous low-dose endotoxin (0.8 ng/kg) or placebo to 11 healthy subjects who had received oral pre-treatment with citalopram (10 mg twice a day) or placebo for five days. The Montgomery-Åsberg Depression Rating Scale, the State and Trait Anxiety Inventory, and a visual analog scale were used to measure depressive and anxiety symptoms and social anhedonia. Serum levels of TNF and IL-6 were measured with immunoassays. Compared to placebo, endotoxin administration increased serum levels of TNF and IL-6, and caused mild depressive-like symptoms, in particular lassitude and social anhedonia. While citalopram pre-treatment had no effect on the innate immune response to endotoxin, it reduced the endotoxin-induced MADRS total score by 50%, with a moderate effect size (Cohen's $d=0.5$). Most of the MADRS total score was due to the Lassitude item, and citalopram pre-treatment specifically reduced endotoxin-induced lassitude with a large effect size (Cohen's $d=0.9$). These results suggest that subchronic pre-treatment with the serotonin-reuptake inhibitor citalopram blunts mood symptoms induced by acute immune system activation with endotoxin without inhibiting the peripheral immune response.

Keywords

Fatigue; Depression; Endotoxin; Cytokines; Citalopram; Antidepressant

1. Introduction

The pathogenesis of depression is incompletely understood. Immune abnormalities, e.g. increased levels of inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6) may play a role (Dowlati et al. 2010). This is supported by the fact that experimentally elevating circulating levels of TNF and IL-6, with e.g. endotoxin, can produce depressive symptoms (Reichenberg et al. 2001; Eisenberger et al. 2009; DellaGioia and Hannestad 2010). Endotoxin administration in humans has been used to study the systemic response to innate immune system activation because doses of 2–4 ng/kg cause

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increases in serum TNF and IL-6 levels similar to those seen in sepsis (Michie et al. 1988; Suffredini et al. 1999). Lower doses (0.4–0.8 ng/kg) cause less pronounced increases in TNF and IL-6 levels, and mild depressive symptoms (Reichenberg et al. 2001; Eisenberger et al. 2009). Rodent data suggest that depressive behaviors produced by immune activation depend on serotonin availability (O'Connor et al. 2009). Consistent with this, depressive symptoms that occur in the context of interferon-alpha (IFN- α) treatment are influenced by polymorphisms in the serotonin transporter gene (Bull et al. 2009; Lotrich et al. 2009) and can be prevented and treated with serotonin-reuptake inhibitor (SRI) antidepressants (Raison et al. 2007; Kraus et al. 2008). No information is available on the effect of serotonin reuptake inhibition on endotoxin-induced depressive symptoms in humans. The goal of this study was to determine whether pre-treatment with citalopram could reduce depressive symptoms produced by acute administration of low-dose endotoxin.

2. Methods

2.1. Design

This is a double-blind, randomized, placebo-controlled, three-way cross-over study in which each subject received oral placebo pre-treatment followed by intravenous (i.v.) placebo administration (Pbo-Pbo condition), oral placebo pre-treatment followed by i.v. endotoxin (Pbo-End condition), and oral pre-treatment with citalopram followed by i.v. endotoxin (Cit-End condition). Each study day was separated by two weeks.

2.2. Subjects

Eleven healthy subjects (five women; mean age 32 ± 9 yrs) were enrolled. Eligibility was based on medical and psychiatric history, review of systems, physical exam, labs and EKG. All subjects provided written, informed consent, and the study was approved by the Yale University Human Investigations Committee.

2.3. Citalopram Pre-Treatment

Subjects received ten capsules of double-blind study medication (10 mg citalopram or placebo) which they took every morning and every evening starting five days before each study day. The last dose was taken on the evening before each session to avoid acute effects of citalopram. Trough levels of citalopram were measured on the morning of the Cit-End session (~12 hours after the last dose).

2.4. Endotoxin Administration

Subjects fasted (except water) from midnight before each session and arrived at the Clinical Neuroscience Research Unit at 8 AM. An i.v. catheter was inserted and 500 ml of normal saline infused to ensure adequate hydration. Baseline ratings and blood draws were performed between 08:30 and 09:00. NIH Clinical Center Reference Endotoxin (0.8 ng/kg) was prepared by the research pharmacist the day before and stored at 4°C. At approximately 9 AM endotoxin or placebo was administered as an i.v. bolus, followed by normal saline to ensure complete delivery. Heart rhythm was continuously monitored. Blood pressure and heart rate were recorded at 5, 10, 15, 20, and 30 minutes after endotoxin administration and every 30 minutes thereafter. Body temperature was measured hourly. Behavioral ratings and blood samples for cytokines were obtained at 1, 2, and 3 hours post-endotoxin/placebo administration.

2.5. Behavioral Ratings

The primary outcome was the Montgomery-Åsberg Depression Rating Scale (MADRS), which was chosen over the Hamilton Depression Rating Scale because it is less oriented

towards somatic symptoms (Demyttenaere and De Fruyt 2003). Secondary outcomes included social anhedonia assessed with a visual-analog scale (VAS; “I want to be alone” vs “I want to be with other people”) and anxiety which was assessed with the State and Trait Anxiety Inventory (STAI).

2.6. Serum Cytokine Assays

Quantikine® quantitative sandwich enzyme immunoassays (R&D Systems, Inc., Minneapolis, MN) were used to measure serum levels of TNF and IL-6. The mean minimum detectable dose is 0.106 pg/ml and 0.039 pg/mL, for TNF and IL-6, respectively. The coefficient of variation was 2% and 1.7%, respectively.

2.7. Statistical Analysis

Outcomes were summarized descriptively and assessed for normality prior to analysis using normal probability plots and Kolmogorov test statistics. The change in each dependent variable (TNF, IL-6, MADRS total, MADRS Lassitude, VAS Social, and STAI) was assessed using a linear model which included treatment (Pbo-Pbo, Pbo-End, Cit-End) and time (0, 60, 120, and 180 minutes; 0 and 120 for MADRS) as within-subject factors, and subject as a clustering factor. Non-normal outcomes were analyzed with the same factors, using a nonparametric approach for repeated-measures data, where the data were first ranked, and then fitted using a mixed effects model with an unstructured variance-covariance matrix and *p* values adjusted for ANOVA-type statistics (ATS). All tests are two-sided and considered statistically significant at $\alpha=.05$. All analyses were performed using SAS, version 9.1 (SAS Institute Inc., Cary, NC). Data are reported as mean $\pm$ standard error of the mean (S.E.M.).

3. Results

No serious adverse events occurred. One subject had nausea and vomiting on the first study day and decided to withdraw. His data were not included in further analyses. All other subjects tolerated the study well. Of the ten remaining subjects, two had no detectable citalopram in their plasma, indicating that they had not taken the capsules as instructed. Because the main outcome was the effect of citalopram pre-treatment on endotoxin-induced symptoms, the data for these two subjects were excluded from final analyses. In the remaining eight subjects (3 women, 5 men) the trough citalopram plasma level was 24.7 ± 5.0 ng/ml, which is lower than that (42 ± 17 ng/ml) achieved in outpatients treated with 20 mg/day (de Mendonca Lima et al. 2005), indicating that steady-state levels were not fully reached. Endotoxin administration caused an increase in heart rate from 63 ± 14 to 89 ± 14 bpm and from 63 ± 12 to 91 ± 10 bpm in the Pbo-End and Cit-End conditions, respectively. There were no significant changes in systolic or diastolic blood pressure or in body temperature.

3.1. Serum Cytokine Levels

At baseline, TNF levels were 1.3 ± 0.2 , 2 ± 0.6 , and 1.4 ± 0.3 pg/ml on the morning of the Pbo-Pbo, Pbo-End, and Cit-End session, respectively. These were not significantly different (all $p > .15$). IL-6 levels were 1.1 ± 0.3 , 1.3 ± 0.4 , and 0.9 ± 0.2 , and there was a trend difference between Pbo-End and Cit-End ($p = .094$), and between Pbo-Pbo and Cit-End ($p = .1$), suggesting that citalopram may reduce IL-6 levels. After endotoxin administration, TNF serum levels increased to a peak level at 2 hrs of 45.2 ± 10.8 and 38.3 ± 6.2 pg/ml in the Pbo-End and Cit-End condition, respectively (Fig. 1A). There was a main effect of endotoxin on TNF levels (ATS = 57.7, num df = 1.5, $p < .0001$), but no effect of citalopram pre-treatment for any of the three timepoints (all $p > .8$). Endotoxin administration had a main effect on IL-6 serum levels (ATS = 41.9, num df = 1.7, $p < .0001$) which peaked at

197.9 ± 47.9 pg/ml and 215.9 ± 54.2 in the Pbo-End and Cit-End condition, respectively (Fig 1B). There was no effect of citalopram pre-treatment on endotoxin-induced IL-6 levels (all $p > .8$). This suggests, within the limits of the small sample size, that citalopram pre-treatment does not inhibit the TNF and IL-6 response to endotoxin.

3.2. MADRS Total Score and MADRS Lassitude

There was a main effect of endotoxin on MADRS total score (ATS = 7.2, num df = 1.9, $p = .0001$). There was a significant difference between Pbo-Pbo and Pbo-End (ATS = 50.2, num df = 1, $p < .0001$), while the difference between Cit-End and Pbo-Pbo was lesser in magnitude (ATS = 4.9, num df = 1, $p = .026$). Consistent with this, there was a significant difference between Pbo-End and Cit-End (ATS = 8, num df = 1, $p = .005$), demonstrating that citalopram pre-treatment reduced the overall magnitude of endotoxin-induced depressive symptoms. The reduction in total MADRS score was ~50% with a moderate effect size (Cohen's $d = 0.5$). Because the MADRS Lassitude item accounted for over 30% of the total MADRS score, we assessed specifically whether citalopram pre-treatment had an effect on this item. In the Pbo-End condition the increase in MADRS Lassitude score from baseline was 1.5 ± 0.3 , while in the Cit-End condition it was 0.8 ± 0.3 (paired $t(7) = 2.4$, $p = .048$), indicating that citalopram pre-treatment reduced endotoxin-induced lassitude by ~50% (Fig. 2A). The effect size of citalopram pre-treatment on endotoxin-induced lassitude was large (Cohen's $d = 0.9$). No other MADRS items were significantly different between Pbo-End and Cit-End. There was no correlation between IL-6 and TNF levels on the one hand, and MADRS total or MADRS Lassitude scores on the other.

3.3. Social Anhedonia

Endotoxin administration caused a reduction in the interest in social interactions, as measured with a VAS (Fig. 2B). There was a main effect of endotoxin on social anhedonia ($F(1,8) = 7.2$, $p = .028$) and a significant condition-by-time interaction ($F(2,15) = 4.8$, $p = .027$). Comparing Pbo-End and Cit-End, there was a trend effect of citalopram pre-treatment blunting endotoxin-induced social anhedonia ($F(1,6) = 4.5$, $p = .078$), and a significant condition-by-time interaction ($F(3,18) = 3.8$, $p = .029$). On the Pbo-End day there was a negative correlation between the change in social anhedonia from 1 to 2 hrs and the change in TNF serum levels ($r = -0.86$, $p = .014$). In other words, the higher the TNF levels, the more social anhedonia subjects reported. There was no correlation on the Cit-End day, and no correlation with IL-6 levels. These data suggest that citalopram may reduce the social anhedonic effect of endotoxin.

3.4. Anxiety

There was no effect of treatment on STAI score (ATS = 0.17, df = 1.7, $p = .8$) and no difference in pair-wise comparisons (all $p > .2$), indicating that endotoxin had no significant effect on anxiety.

4. Discussion

This is the first study in humans showing that a serotonin reuptake inhibitor, citalopram, can reduce endotoxin-induced depressive symptoms, as measured by the MADRS, our primary endpoint. Most of the preventive effect of citalopram was due to an inhibition of endotoxin-induced increases in the lassitude item, which measures fatigue and motivation. Citalopram pre-treatment also had a trend level effect inhibiting endotoxin-induced social anhedonia, which is consistent with findings by Eisenberger (Eisenberger et al. 2010). Lastly, endotoxin-induced social anhedonia correlated with serum TNF levels

Immune system activation (with IFN- α or endotoxin) tends to cause neurovegetative rather than cognitive symptoms of depression (Capuron et al. 2009; DellaGioia and Hannestad 2010). Our results, which show that most of the increase MADRS score is due to changes in fatigue and motivation, are consistent with this. Fatigue is a common symptom of depression (Nutt et al. 2007). The neurobiology of fatigue is thought to involve dopamine and serotonin (Meeusen et al. 2006; DeLuca et al. 2009). Our findings indicate that citalopram, which has negligible effects on neurotransmitters other than serotonin, can reduce endotoxin-induced fatigue.

Citalopram pre-treatment had no inhibitory effect on the TNF and IL-6 response to endotoxin. This may not be generalizable due to the small sample size, however, in our subjects, because of the lack of any difference in the TNF and IL-6 response to endotoxin, it is unlikely that the inhibitory effect of citalopram on endotoxin-induced depressive symptoms occurs in the periphery. Rather, citalopram pre-treatment may render subjects less susceptible to developing endotoxin-induced depressive symptoms through its effect on serotonin reuptake in the brain. Inflammatory cytokines induce expression of the enzyme indoleamine 2,3 dioxygenase (IDO), which converts tryptophan to kynurenine, leaving less tryptophan available for serotonin synthesis (Muller and Schwarz 2007). Interference with serotonin turnover is believed to be one of the mechanisms of IFN- α -induced depression (Capuron et al. 2003; Raison et al. 2010). Our results suggest that IDO-induction may be one pathway through which endotoxin produces depressive symptoms, since these symptoms can be reduced by serotonin reuptake inhibition.

Another mechanism by which peripheral inflammation might produce depressive symptoms is through the induction of neuroinflammation (McNally et al. 2008). In mice the SRI paroxetine prevents neuroinflammation (Chung et al. 2010), indicating that serotonin reuptake inhibition may have anti-inflammatory effects in the brain. Interestingly, citalopram administration for five days was associated with a reduction in baseline IL-6 levels at a trend level (albeit no changes in TNF levels). This is consistent with rodent studies showing that antidepressants have anti-inflammatory effects (Xia et al. 1996; Roumestan et al. 2007).

There are several limitations to this study. First, the small sample size makes the study prone to both Type 1 and Type 2 error. That is, our positive findings, e.g. that citalopram inhibits endotoxin-induced lassitude, may not be generalizable. More importantly, we cannot conclude with certitude that citalopram pre-treatment *does not* inhibit the TNF and IL-6 response to endotoxin. Second, although the MADRS lassitude item likely measures fatigue and motivation, no formal fatigue scale was used. Third, we measured levels of TNF and IL-6, but not of other cytokines involved in the immune response to endotoxin.

In summary, our data suggest that subchronic treatment with the serotonin reuptake inhibitor citalopram reduces depressive symptoms produced by acute, low-dose endotoxin administration in human subjects. It remains to be shown whether longer duration and/or higher doses of citalopram, or pre-treatment with medications that inhibit reuptake of norepinephrine and dopamine can further reduce endotoxin-induced symptoms. The human low-dose endotoxin model reliably induces fatigue and may therefore be useful in studies of novel agents that target fatigue.

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Fig. 1. The inflammatory cytokine response to endotoxin

(A) Endotoxin administration caused an increase in serum levels of TNF, and this increase was not blunted by citalopram pre-treatment.

(B) Endotoxin administration caused an increase in serum levels of IL-6, and this increase was not blunted by citalopram pre-treatment. Error bars denote S.E.M.

Fig. 2. The behavioral response to endotoxin

(A) Endotoxin administration caused an increase in locomotion which was blunted by ~50% by pre-treatment with active citalopram (Cit-End) compared to placebo pre-treatment (Pbo-End).

(B) Endotoxin administration caused social anhedonia which was blunted by pre-treatment with citalopram at a trend level. Error bars denote S.E.M.