Tumor Necrosis Factor Antagonism Normalizes Rapid Eye Movement Sleep in Alcohol Dependence

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Background: In alcohol dependence, markers of inflammation are associated with increases in rapid eye movement (REM) sleep, which is thought to be a prognostic indicator of alcohol relapse. This study was undertaken to test whether blockade of biologically active tumor necrosis factor-α (TNF-α) normalizes REM sleep in alcohol-dependent adults.

Methods: In a randomized, placebo-controlled, double-blind, crossover trial, 18 abstinent alcohol-dependent male adults received a single dose of etanercept (25 mg) versus placebo in a counterbalanced order. Polysomnographic sleep was measured at baseline and for 3 nights after the acute dose of etanercept or placebo.

Results: Compared with placebo, administration of etanercept produced significant decreases in the amount and percentage of REM sleep. Decreases in REM sleep were robust and approached low levels typically found in age-comparable control subjects. Individual differences in biologically active drug as indexed by circulating levels of soluble tumor necrosis factor receptor II negatively correlated with the percentage of REM sleep.

Conclusions: Pharmacologic neutralization of TNF-α activity is associated with significant reductions in REM sleep in abstinent alcohol-dependent patients. These data suggest that circulating levels of TNF-α may have a physiologic role in the regulation of REM sleep in humans.

Sleep disturbance is one of the most prominent complaints of alcoholic patients, with more than 70% of alcohol-dependent persons reporting sleep problems that fail to resolve over the course of abstinence (1,2). Moreover, poor sleep predicts risk of relapse in recovering alcohol-dependent persons (3). It has been further demonstrated that abnormal increases in the percentage of rapid eye movement (REM) sleep are prospectively associated with risk of alcoholic relapse, which suggests that disturbances in REM sleep have prognostic implications in alcohol recovery (4,5). The neurobiological mechanisms that underlie the increases of REM sleep seen during alcohol dependence are not known, although recent evidence suggests that the production of tumor necrosis factor-α (TNF-α) and possibly other markers of inflammation may have a role. Basic observations in rodents demonstrate that TNF-α acts physiologically to regulate sleep (6,7). In alcohol dependence, it has been found that mononuclear cell production of interleukin-6 (IL-6) and TNF-α correlates with REM sleep amounts (8). Furthermore, in studies that temporally profile circulating levels of proinflammatory cytokines across the night, such increases correlate with increases in REM sleep amounts during the subsequent sleep period (9). Taken together, these studies suggest that circulating levels of TNF-α may have a negative influence on the homeostatic regulation of sleep and underlie the relative increases in REM sleep observed in alcohol dependence (10,11).

Given these associations between TNF-α and REM sleep amounts, we hypothesized that blockade of biologically active TNF-α by the antagonist etanercept (Enbrel; Immunex, Thousand Oaks, California) might normalize REM sleep in alcohol-dependent adults. Etanercept is a recombinant human protein composed of two binding domains of the p75 TNF receptor II (TNFRII) fused to the Fc portion of an immunoglobulin molecule (12). It binds specifically to TNF-α in circulation, preventing TNF binding to cell surface receptors, thereby intercepting the inflammatory cascade. Etanercept is an Food and Drug Administration–approved medication for the treatment of rheumatoid arthritis and is given in an injectable form at a subcutaneous (SC) dose of 25 mg (13). Using a randomized, double-blind, placebo-controlled, crossover design, this study tested the effects of etanercept on REM sleep amounts in alcohol dependence. Secondarily, we also explored the effects of etanercept on measures of sleep continuity and sleep architecture.

Methods and Materials

Design Overview

This randomized, placebo-controlled, double-blind, crossover trial allocated abstinent alcohol-dependent adults to receive either a single dose of etanercept (25 mg) versus placebo in a counterbalanced order. After completion of psychiatric interviews, medical evaluations, and an adaptation night in the sleep laboratory with screening for sleep apnea and nocturnal myoclonus as previously described (10,11), eligible subjects were randomized to placebo or drug as a first session. Following baseline assessment of sleep, subjects received either etanercept or placebo and underwent 3 nights of sleep evaluation. After at least 2 weeks of washout, participants underwent a second baseline sleep evaluation and were administered a second dose of either etanercept or placebo, depending on which drug they had received during the first session. Subjects then underwent by an additional 3 nights of sleep evaluation.

Control subjects were not eligible for enrollment per University of California, Los Angeles (UCLA) Internal Review Board (IRB) guidelines. Hence, to determine whether changes in REM

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sleep following etanercept administration generated levels comparable to normal volunteers, age- and sex-comparable laboratory control subjects with no psychiatric disorder ($n = 119$ (14) were identified from prior studies (9–11,15–18).

Procedures

Participants who responded to the advertisement ($N = 55$) between October 2006 and June 2007 underwent assessment phases as previously described (9–11,15–17). Of the 55 subjects evaluated, 14 were excluded because of medical issues (e.g., positive tuberculin skin test), body mass index (BMI) > 30, or both; 5 due to comorbid psychiatric disorders (e.g., current major depressive disorder); 6 for failure to maintain abstinence for 2 weeks before testing; and 4 due to other comorbid, current substance dependence. Of the remaining 25 subjects, 4 additional subjects declined to participate. Details regarding recruitment methods, as well as inclusion and exclusion criteria are found in Supplement 1.

Twenty-one subjects were randomized and admitted to the UCLA General Clinical Research Center for sleep evaluation (Figure 1). Given the experimental nature of this study and concern about adverse side effects associated with the administration of etanercept in this population, IRB guidelines specified that the first five participants be directly allocated to receive active drug followed by placebo. However, drug allocation remained blinded for these five subjects, as well as for the staff who were charged with assessing them or scoring sleep records; only the study physician (M.R.I.), the statistician who generated the randomization schedule, and the pharmacist were aware of active drug assignment. After no adverse events were observed, the remaining 16 subjects were randomly allocated to etanercept or placebo in a counterbalanced order, taking into account the prior schedule. Before administration of etanercept or placebo, three subjects were excluded because of recent use of other substances (i.e., positive urine tests). In the interval between sessions, an additional four participants reported using alcohol and were excluded. Procedures for polysomnographic assessment (19) and assay of soluble TNFRII (sTNFRII) and soluble TNF-α are previously described (9) and also found in Supplement 1.

Statistical Analysis

Given correlational evidence that proinflammatory cytokines are associated with increases in REM sleep amounts (9), the primary outcome of interest was change in amounts of REM sleep following administration of etanercept versus placebo. To determine whether REM sleep amounts before and after etanercept differed from levels found in age and sex comparable laboratory control subjects ($n = 119$), whose electroencephalographic sleep measures had been previously reported (9–11,15–18), planned comparisons were conducted. Secondary exploratory outcomes were changes in sleep continuity measures, along with other measures of sleep architecture. We estimated on the basis of our prior correlational data (9), as well as findings that have examined the effects of another TNF-α antagonist, infliximab, on sleep architecture (20), that the enrollment of 15 patients would provide the study with a statistical power of more than 80% ($\alpha = .05$) to detect a difference in REM sleep amounts.

The general effects of drug administration were assessed using a mixed models condition (etanercept vs. placebo) × night (baseline, experimental nights 1–3) × order (etanercept, first or second session) repeated-measures analysis of variance (rANOVA) for REM sleep (amounts, percentage). Secondary covariate analyses separately examined the contribution of liver

enzymes, predrug TNF-α levels, direct allocation assignment, and completer status on the effects of etanercept on REM sleep. Further exploratory analyses examined the effects of etanercept on sleep continuity outcomes (i.e., total sleep time, sleep latency, sleep efficiency), other sleep measures (i.e., Stages 1–4 sleep, REM density, REM duration), and the relationship between circulating sTNFRII levels and REM sleep. All analyses used an intention to treat approach with inclusion of subjects who had at least one session.

Results

Subjects fulfilled criteria for alcohol dependence in partial remission as determined by Structured Clinical Interview for DSM-IV and had a mean age of 41.4 years [SD = 9.5], education level of 13.5 years [SD = 1.8], and BMI of 25.7 [SD = 4.0]. Of the sample, 50.0% were nonwhite, and 72.2% were current smokers. All subjects were male and had liver enzymes in the laboratory normal range: aspartate aminotransferase [SGPT; mean, 28.3 U/L ± 16.8], alanine aminotransferase [SGOT; mean, 29.4 U/L ± 24.0], and bilirubin [mean, .8 μmol/L ± .4]. Pre-etanercept levels of circulating TNF-α were detectable in all subjects [mean, 1.0 pg/mL ± .4].

Amount and percentage of REM sleep showed acute decreases following administration of etanercept as compared to placebo. Among the subjects who completed at least one session, significant condition × night interactions were found for amounts and percentage of REM sleep [$F(3,90.0) = 2.6$, $p = .05$; $F(3,88.4) = 2.6$, $p = .05$; Figure 2], with decreases of REM sleep occurring during the night immediately following drug adminis-
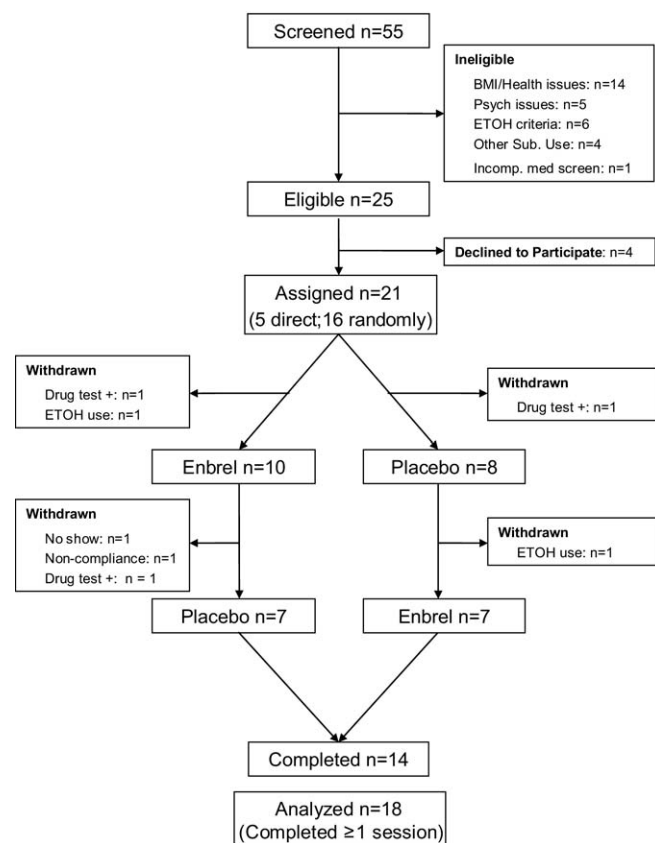

Figure 1. Participant flow and distribution of subjects in study. BMI, body mass index; ETOH, ethyl alcohol.

tration ($p < .05$; 95% confidence interval [CI] of difference vs. placebo condition: $-.6$ to -7.7). Furthermore, this decrease of percentage of REM sleep approached levels found in age- and sex-comparable laboratory control subjects ($n = 119$). At baseline, percentage of REM sleep was significantly higher in the alcohol-dependent subjects compared with laboratory controls ($p < .01$; 95% CI: 23.4–30.4). However, after a single dose of etanercept, percentage of REM sleep decreased in the alcohol-dependent participants and was statistically similar to levels found in control subjects ($p = .08$; 95% CI: 20.1–27.2). The effects of a single dose of etanercept on REM sleep were transient, and amount and percentage of REM sleep returned to levels found in the placebo condition during the second and third postinjection nights. Inclusion of liver enzyme values or predrug levels of circulating TNF-α, as covariates did not alter the effect of etanercept on REM sleep. In addition, given that the first five subjects were directly allocated to active drug, additional analyses were conducted in which the direct allocation assignment was treated as a covariate; results were similar for amounts and percentage of REM sleep [$F_{(3,88.1)} = 2.6$, $p = .059$; $F_{(3,88.7)} = 2.6$, $p = .058$]. Likewise, given that 14 subjects completed both sessions, additional analyses were performed in which completer status was treated as a covariate; again, results were similar for amounts and percentage of REM sleep [$F_{(3,87.8)} = 2.6$, $p = .06$; $F_{(3,88.4)} = 2.6$, $p = .06$]. Differences in REM sleep were similar in those who received etanercept during the first versus second session. Finally, other measures of sleep continuity, sleep architecture, and REM density and duration were not altered (all $ps > .05$).

Responses of REM sleep to a single dose of etanercept showed individual variability, and further analyses explored whether individual differences in biological active drug as indexed by circulating levels of sTNFRII were associated with percentage of REM sleep. Circulating concentrations of sTNFRII obtained 24 hours after drug administration were negatively correlated with percentage of REM sleep (Figure 3; $y = -8262.4x + 382518$; $R^2 = .48$; $r = -.69$, $p < .01$).
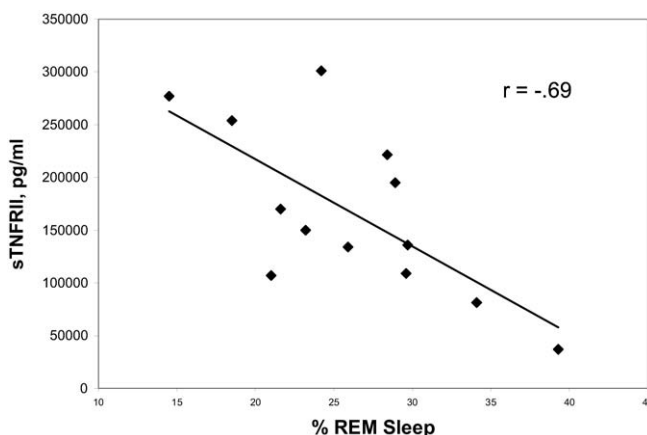
Figure 3. Association between biologically active etanercept as indexed by circulating levels of sTNFRII and percentage of rapid eye movement (REM) sleep during the first night after drug administration.

Discussion

Although the inflammatory response to sleep disturbance has been described (21,22), much less is known about the reciprocal action of proinflammatory cytokines on regulation of sleep in humans. This randomized, placebo-controlled study demonstrates that neutralizing TNF-α activity is associated with a significant reduction of REM sleep in abstinent alcohol-dependent patients. The reduction in percentage of REM sleep was robust; levels of REM sleep approached those typically seen in healthy volunteers without a history of alcohol dependence. Moreover, individual biological variability in the degree of TNF antagonism, as reflected by circulating levels of sTNFRII, correlated with declines in REM sleep. Taken together, these data support the hypothesis that circulating levels of TNF-α may have a physiologic role in the regulation of REM sleep amounts in humans.

These observations further suggest that inhibition of proinflammatory cytokine signaling might represent a viable strategy for targeting sleep disturbance, especially in patients with evidence of increased inflammatory activity. Antagonists of TNF-α (i.e., etanercept, infliximab) reportedly reduce depressive symptoms in the context of treating psoriasis (23). Moreover, one prior study reported that acute administration of infliximab resulted in improvements in sleep continuity and depth in rheumatoid arthritis patients, although this trial was uncontrolled and did not include a placebo condition (20).

Studies in laboratory animals demonstrate that cytokine-induced sickness behaviors, of which sleep disturbance is one component, can be reversed by administering specific cytokine antagonists (e.g., IL-1ra) or anti-inflammatory cytokines (e.g., IL-10) directly into the brain (24). Whereas in animals, proinflammatory cytokines are thought to promote increases of NREM sleep with reciprocal decreases of REM sleep (6), other data show that mice lacking the TNF 55 kD receptor that signals the effects of TNF-α have decreases of NREM as well as REM sleep (25). In six persons who were infected with human immunodeficiency virus (HIV), circulating levels of TNF-α were found to correlate with delta sleep amplitude as assessed by spectral analyses (26). However, these data contrast with other findings showing a relationship between inflammatory activity and increases of REM, but not delta, sleep in humans (8,9,27,28). In this study, the absence of an effect of TNF antagonism on delta sleep
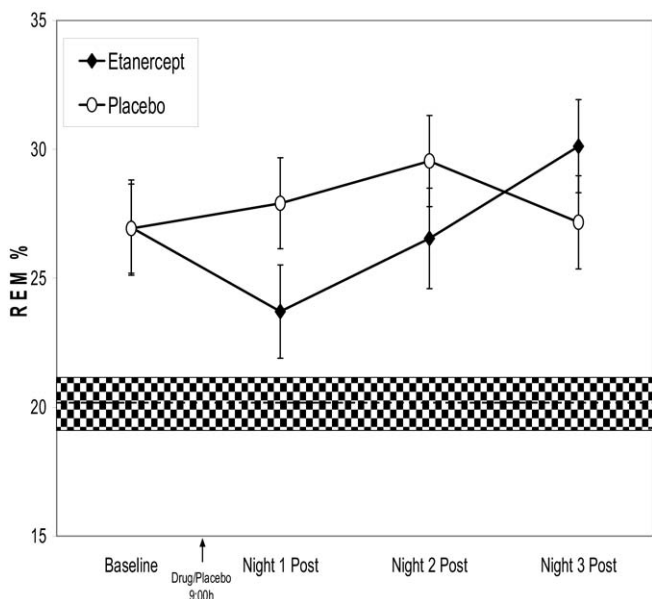

Figure 2. Changes in percentage of rapid eye movement (REM) sleep from baseline to three nights postdrug (etanercept ○ vs. placebo ●) in abstinent alcohol-dependent adults. The shaded area indicates the percentage of REM sleep in age comparable normal volunteers.

is not surprising, given our prior findings that alcohol dependence may be associated with a defect in the homeostatic regulation of delta sleep, because alcohol-dependent patients fail to show an increase in delta sleep during recovery sleep after sleep deprivation (10).

The mechanisms that bridge the link between peripheral levels of TNF-α and abnormal REM sleep amounts in alcohol dependence are not fully understood. However, prior data have found that chronic alcohol consumption is associated with increases in monocyte production, as well as circulating levels, of TNF-α (9,29,30). Furthermore, acute and chronic ethanol exposure induces production of TNF-α by liver Kupffer cells, which contribute to a significant portion of systemic TNF-α (30). In turn, circulating TNF-α triggers rapid (within minutes) increase in the expression of brain TNF-α (31); TNF receptors are thought to transport circulating TNF-α across the blood-brain barrier (32,33). Whereas liver and systemic cytokine responses decline within days after ethanol exposure, brain expression of TNF-α appears to be long lasting (31). Indeed, persistent elevations of brain TNF-α could explain abnormal REM sleep amounts in abstinent alcohol-dependent persons, even though circulating levels of TNF-α are comparable to levels found previously in normal volunteers (9).

The action of acute TNF antagonism on REM sleep was found only during the first night after drug administration, with REM sleep returning to abnormally elevated levels during the second and third nights after administration of etanercept. Given that a single subcutaneous dose of etanercept has a mean half-life of over 100 hours, it is not known why the action of TNF antagonism was short in duration. Because of the persistence of high levels of long-lived TNF/etanercept complexes in the circulation, it is not possible to assess the extent of TNF antagonism by determining circulating levels of free TNF-α post-etanercept. Given the variability of concentrations of circulating sTNFRII observed among subjects post-etanercept, initial bioavailability of the drug may be important. Future studies should assess whether administration of etanercept for a longer period will be associated with a more marked and durable improvement in REM sleep and possibly measures of sleep continuity. Twice weekly administration of etanercept for 3 weeks has been found to induce marked decreases in sleepiness in sleep apnea patients, although that study did not report polysomnographic measures (34).

Limitations of this study include small sample size and sex, along with assessment of sleep solely by visually scoring approaches without additional inclusion of spectral analyses. Despite these limitations, this controlled experimental study showed that TNF-α is a mediator of REM sleep in humans, which substantially extends previous observational studies and represents an important step in ongoing investigations to determine the mechanisms that underlie disturbed sleep in patients with substance use disorders. Further interventional studies that attempt to correct these abnormalities in inflammatory signaling will promote the understanding of the pathophysiologic mechanisms that contribute to sleep disturbance in alcohol dependence and, possibly in other morbid inflammatory conditions.

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