TNF-alpha Modulation for Treatment of Alzheimer's Disease: A 6-Month Pilot Study

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Abstract and Introduction

Abstract

Context: Current pharmacologic treatments for Alzheimer's disease (AD) do not prevent long-term clinical deterioration. Tumor necrosis factor (TNF)-alpha, a proinflammatory cytokine, has been implicated in the pathogenesis of AD.

Objective: To investigate the use of a biologic TNF-alpha inhibitor, etanercept was given by perispinal extrathecal administration for the treatment of AD.

Methods: This was a prospective, single-center, open-label, pilot (proof-of-concept) study, in which 15 patients with mild-to-severe AD were treated for 6 months. We administered etanercept, 25-50 mg, once weekly by perispinal administration. Main outcome measures included the Mini-Mental State Examination (MMSE), the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog), and the Severe Impairment Battery (SIB).

Results: The average age of our patient population was 76.7. The mean baseline MMSE was 18.2 (n = 15); the mean baseline ADAS-Cog was 20.8 (n = 11); and the mean baseline SIB was 62.5 (n = 5). There was significant improvement with treatment, as measured by all of the primary efficacy variables, through 6 months: MMSE increased by 2.13 ± 2.23, ADAS-Cog improved (decreased) by 5.48 ± 5.08, and SIB increased by 16.6 ± 14.52.

Conclusion: An increasing amount of basic science and clinical evidence implicates inflammatory processes and resulting glial activation in the pathogenesis of AD. This small, open-label pilot study suggests that inhibition of the inflammatory cytokine TNF-alpha may hold promise as a potential approach to AD treatment. Further study in randomized, placebo-controlled clinical trials is merited.

Introduction

Inflammatory immune mechanisms play a central role in the causation of Alzheimer's disease (AD).[1-5] Tumor necrosis factor (TNF)-alpha, a proinflammatory cytokine, the "master regulator" of the immune response, is the key initiator of immune-mediated inflammation in multiple organ systems, including the brain.[6] Scientific evidence identifying TNF-alpha involvement in the pathogenesis of AD began accumulating a decade ago in experimental models. In vitro, with use of a human monocytic cell line, beta amyloid was found to stimulate secretion of TNF-alpha.[7] TNF-alpha plus gamma-interferon was found to induce beta-amyloid production.[8] Beta amyloid was shown to stimulate microglial inflammatory pathways, resulting in neurotoxicity mediated by TNF-alpha generated by reactive microglia and monocytes.[9] Clinical evidence followed, with a central place for TNF-alpha in AD pathogenesis suggested by demonstration of 25-fold elevated levels of TNF-alpha in the cerebrospinal fluid of patients with AD,[10] and the finding that increased cerebrospinal fluid TNF-alpha levels correlated with clinical deterioration.[11] In 2005, the evidence supporting TNF-alpha involvement in AD accelerated, including identification of a greater risk for AD in an Australian population associated with a polymorphism in the promoter region of the TNF gene.[12]

Increasing amounts of laboratory evidence implicate TNF-alpha in inflammatory molecular mechanisms producing neurototoxicity, neuronal death, or neuronal dysfunction involving both TNF-glutamate[13-17] or TNF-amyloid interactions.[18-22] In a brain slice culture model, TNF-alpha was found to potentiate glutamate neurototoxicity, with TNF-alpha and glutamate acting synergistically to induce neuronal cell death.[13] Stimulation of microglial metabotropic glutamate-2 receptors on rat primary microglia was found to induce TNF-alpha release, and contribute to microglial neurototoxicity.[14] In cultured hypothalamic cells, glutamate was found to induce the expression and release of TNF-alpha, which was postulated to be potentially related to physiologic regulation of sleep and wakefulness.[15] TNF-alpha both directly affects glutamatergic synaptic transmission, increasing AMPA receptors on synapses, and modulates synaptic plasticity.[16,17] Of particular relevance to memory impairment in AD, beta-amyloid inhibition of long-term potentiation appears to be mediated by TNF-alpha.[23]
Substantial laboratory evidence implicates beta-amyloid-induced neuroinflammation with neurotoxicity, and this appears to be an early event in neurodegeneration. Experimental models using beta-amyloid-stimulated murine microglia suggest that beta-amyloid-induced neuronal death may be mediated by synergy between TNF-alpha and glutamate-induced neurotoxicity. In addition to TNF-alpha, beta amyloid upregulates other inflammatory mediators in the brain, including interleukin (IL)-1 beta, IL-6, nitric oxide, and inducible nitric oxide synthase. Increasing evidence suggests that microscopic inflammation resulting from the release of inflammatory cytokines, including TNF-alpha, by amyloid-beta-activated microglia plays a central role in the neurotoxicity that occurs in AD. This hypothesis suggests that specific anti-inflammatory agents that downregulate this inflammatory process could potentially be of therapeutic benefit in AD.

Therapeutic agents that selectively inhibit the biologic activity of TNF-alpha have recently become available for human use. One of these is etanercept, a dimeric fusion protein that is produced with recombinant DNA technology and composed of 934 amino acids with a total molecular weight of 150,000 d. It consists of a fragment of the human 75-kd (p75) TNF receptor linked to the Fc portion of human immunoglobulin (Ig)G1. Etanercept binds specifically to TNF and blocks its interaction with cell-surface TNF receptors. By avidly binding excess TNF, etanercept functions as an extraordinarily potent TNF antagonist. Because of the known role of inflammation in AD pathogenesis, etanercept has been suggested as a possible therapeutic agent for AD. It is approved by the US Food and Drug Administration (FDA) for the treatment of rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, and psoriasis, with pilot studies and basic science investigations suggesting possible utility for a variety of neurologic disorders.

Pilot studies ("proof-of-concept" studies) in small numbers of patients are a common starting point for new therapeutic approaches. While not providing the same degree of robust scientific evidence offered by randomized, double-blind, placebo-controlled trials, pilot studies are nevertheless useful for helping to define the feasibility of a new scientific approach, and may yield valid statistical data if carefully designed, even with a minimum number of subjects. Indeed, the era of biologic anti-TNF-alpha therapy for the treatment of rheumatoid arthritis was ushered in by an open-label, uncontrolled, nonrandomized pilot study published in The Lancet in 1994 involving only 7 patients.

To investigate the feasibility of using etanercept for the treatment of AD, we initiated a 6-month, open-label pilot study.

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Patients and Methods

We recruited patients residing in the community who had previously been diagnosed with AD by a board-certified neurologist and were clinically declining despite treatment, without age restriction, for inclusion into a 6-month, open-label clinical trial. Inclusion required that the patient be accompanied by a reliable caregiver and have previously performed magnetic resonance imaging (MRI) or a computed tomographic (CT) scan consistent with a primary diagnosis of AD. All participants met the NINCDS-ADRDA Criteria for probable AD; in addition, all met the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for AD. Patients were excluded if they had any of the following: active infection, multiple sclerosis (or any other demyelinating disorder), vascular dementia, clinically significant neurologic disease other than AD or a score greater than 4 on the modified Hachinski Ischemic Rating Scale, pregnancy, uncontrolled diabetes mellitus, tuberculosis, history of lymphoma, or congestive heart failure. Also excluded were female subjects who were premenopausal, fertile, or not on acceptable birth control; and patients with a white blood cell count < 2500 cells/mm³, hematocrit < 30, or a platelet count < 100,000 cells/mm³. In addition, study eligibility required the dose of all central nervous system (CNS)-active medications to be unchanged in the 4 weeks before study initiation and during the entire course of the clinical trial.

Methods

Patients received etanercept (Immunex Corp., Seattle, Washington) as a solution in sterile water given by midline interspinous injection in the posterior neck, via a 27-gauge needle, and were then placed in the Trendelenburg position for 5 minutes, with placement of the head below horizontal. The total dose ranged from 25 mg to 50 mg per week on an open-label basis, with an initial dose of 25 mg once per week. Interspinous injection was extrathecal, into the area between 2 adjacent spinous processes. The 6-month trial was approved by a central institutional review board. Written informed consent was obtained from the patient's legally responsible caregiver in every case, and from all patients who were capable of consent.

The study was funded solely by the principal investigator; the families of the study subjects supplied the etanercept for their family members. Data analysis was performed by an independent statistician; these data are stored at the clinic of the principal investigator.

Efficacy Variables

The primary efficacy variables were the change from baseline in 3 standard measures of cognition: the AD Assessment Scale-Cognitive subscale (ADAS-Cog), the Severe Impairment Battery (SIB), and the Mini-Mental State Examination (MMSE). Additional selective neurocognitive tests were performed, and are being reported separately.
The ADAS-Cog is a multi-item instrument of known reliability used to quantitate overall cognitive function and has been extensively validated in longitudinal cohorts of patients with AD.[37] It is widely used to measure the clinical efficacy of medications used to treat patients with mild-to-moderate dementia. It examines selected aspects of cognitive performance, including elements of memory, orientation, attention, reasoning, language, and praxis. Scores range from 0 to 70. In contrast to most measures of cognitive function, higher scores indicate greater cognitive impairment; therefore, a negative change indicates clinical improvement.

The SIB is a widely used, reliable, and extensively validated instrument specifically designed to measure cognitive function in individuals with moderate-to-severe dementia, a population in which the ADAS-Cog is of diminished usefulness.[38] It is a 51-item scale that assesses social interaction, orientation, memory, language, visuospatial ability, attention, praxis, and construction. Scores range from 0 (greatest impairment) to 100.

The MMSE is a widely used formal cognitive assessment measure used in routine clinical practice.[39] Patients may score in a range from 0 to 30, with lower values indicating reduced cognitive function. The test measures patient orientation to time and place, recall ability, short-term memory, and mental arithmetic. The MMSE is often used to classify a study population with regard to the degree of their baseline cognitive impairment, and may also be used to characterize the rate of disease progression.[39]

Patients were assessed at baseline (treatment day 0) and monthly thereafter. All patients were assessed with the MMSE. Patients with mild and moderate AD were assessed with the ADAS-Cog. Patients with more severe dementia (defined by a baseline ADAS-Cog score above 30 or inability to perform the ADAS-Cog) were assessed with the SIB. The results were analyzed with only the observed values included, without replacing the missing values (observed-cases analysis).

Measures of safety included measurement of vital signs and recording of adverse events.

Statistical Analysis

The changes from baseline in MMSE, ADAS-Cog, and the SIB are considered the primary outcome measures at the end of the 6-month follow-up assessment. Mixed Model Linear Regression (MMLR) analyses were used to assess improvement in disease over time, as evaluated by the 4 outcome measures. In each analysis, time (baseline, 1 month, 2 months, 3 months, 4 months, 5 months, and 6 months) was entered as a fixed variable. The models were also specified with random intercepts, as the participants in this study varied across the spectrum of severity at baseline because recruitment was not limited to a range of severity. Missing data points are treated as missing and are not estimated; this was an observed data analysis.

Data were analyzed with statistical analysis software SPSS (Version 11.0.3 for Mac OS X, SPSS Inc., Chicago, Illinois), with a P value less than .05 indicative of statistical significance.

Results

Study Population and Dosage

All data from all patients (n = 15) who completed at least 1 follow-up evaluation time point were analyzed. All 15 patients completed the first 6 months of treatment. One patient whose dementia was borderline between moderate and severe was assessed with both ADAS-Cog and SIB, in addition to MMSE. The baseline characteristics of the 15-patient study population are presented in Table 1. The average dosage for the study cohort was 32 ± 12 mg/week (n = 15). The CNS-active medications used by the studied patients, in addition to etanercept, memantine, or a cholinesterase inhibitor, included 3 patients on stable doses of antidepressants, 1 on stable doses of risperidone, 1 on stable doses of gabapentin, and 1 on stable doses of olanzapine throughout the trial and for at least 1 month before study initiation (see Table 1).

Primary Efficacy Variables

The baseline scores and the results through 6 months for MMSE, ADAS-Cog, and SIB, are shown individually in Figures 1, 2, and 3, respectively, and in Table 2, collectively.
Figure 1.
Change in Mini-Mental State Examination (MMSE) from baseline to 6 months following initiation of etanercept use.

Figure 2.
Change in Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog) from baseline to 6 months following initiation of etanercept use.
Safety and Tolerability

During the 6-month study period, the following adverse events were observed in the study population: upper respiratory infection (n = 1), hemarthrosis subsequent to an error in warfarin dosage (n = 1), confusion (n = 2), discomfort at the etanercept injection site (n = 1), maculopapular rash on the upper trunk (n = 1), benign skin tumor (keratoacanthoma; n = 1), elevation of blood pressure requiring change in medication (n = 1), and intertrigo (n = 2). One study participant, an 80-year-old woman with severe dementia (baseline MMSE at study initiation of 2) and a history of hypertension and supraventricular tachycardia, died suddenly during the last week of the clinical trial, without obvious cause. Her cognition had improved with etanercept (MMSE had improved to 6 at 24 weeks, measured 1 week before her death). She had been taking multiple medications concurrently with weekly etanercept, including memantine, mirtazapine, tolterodine, and gabapentin, without change in dosage during the clinical trial. An autopsy was not performed.

Case Report

S8 is an 82-year-old, right-handed man who presented with effortfulness of speech, word-finding impairment, and difficulty remembering names of people and places approximately 10 years ago. Antidepressant medication was prescribed. An examination in 1997 revealed numerous deficits involving naming, attention, memory, verbal comprehension, and visuospatial skills. His speech, according to his wife, included paraphasic errors and incorrect word sequencing. His MMSE score in 1997 was 19. Results of MRI of the brain performed in 1996 and 1997 were normal except for mild, diffuse atrophy. Single-photon emission computed tomography (SPECT) of the brain in 1996 showed hypoperfusion in the right parietal region. SPECT of the brain in 1998 and 2001 demonstrated severe hypoperfusion in the posterior parietal cortex bilaterally, thought most likely to represent advanced AD. History includes pacemaker placement and hypercholesterolemia. Thyroid-stimulating hormone and vitamin B12 level were within normal limits.

Despite treatment with donepezil 10 mg/day, begun in 1998, the patient's cognitive status continued to decline. Repeat cognitive testing in 2001 included an MMSE score of 11, with evidence of apraxia as well as progressive impairments of gross cognitive functioning, attention, concentration, language skills, visuospatial abilities, verbal memory, and executive functioning. Because of cognitive worsening, donepezil was replaced with galantamine in 2001. By early 2003, the MMSE score declined to 7, and memantine was added. Despite combination therapy with galantamine 24 mg/day and memantine 10 mg orally twice daily, cognitive function deteriorated relentlessly, with MMSE scores falling to 4 and 0 by September 2004 and January 2005, respectively.

In March 2005, after informed consent, he was begun on weekly doses of etanercept 25 mg given by perispinal...
administration. After 1 month, his speech was more fluent and daily activities, such as removing his jacket and shoes, were improved. After 5 months, he was more aware of his surroundings, more interactive with others, and better able to speak. His functional ability to perform daily activities, as measured by the use of the Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory modified for severe dementia (ADCS-ADLsev), a standardized and validated structured questionnaire, was 20 at baseline, improved to 23 at 3 months, 26 at 4 months, 27 at 5 months, and 27 at 6 months. The patient's SIB and MMSE scores markedly improved with treatment, with MMSE improving from 0 to 4, and SIB improving 35 points (Figure 4). This improvement in SIB was the highest seen in the study cohort.

Figure 4.
SIB and MMSE improvements following initiation of weekly perispinal etanercept in one patient with severe AD.

Discussion

The unmet medical need in AD is substantial, because AD is invariably progressive, and none of the FDA-approved medications for AD is able to prevent continuing cognitive decline over the long term. The majority of AD studies are of 6 months' duration, because during this period the placebo-treated control group characteristically declines measurably, when assessed with the standard AD instruments consisting of ADAS-Cog, MMSE, and, for more severely affected patients, SIB, and the treatment effect, even if small, is therefore thrown into sharper relief.

In contrast to the standard cognitive decline one would historically expect to see during a 6-month observation period, the data of this study suggest that the 15-patient cohort treated with ongoing weekly perispinal etanercept experienced a sustained and significant improvement in cognition, as documented by each of the primary efficacy variables, MMSE, ADAS-Cog, and SIB. The cohort's improvement is in contrast to the known decline of ADAS-Cog by 7 points per year, and the average decline in MMSE of 3.3 points per year, which occurs in untreated populations. Nonetheless, the small study size and uncontrolled nature of the treatment point to the need for randomized controlled trial data to confirm the findings of this study. In particular, in larger studies it may be possible to determine whether any patient characteristics may help in patient selection. This study suggests that patients with AD who have a wide range of severity may benefit, but larger controlled studies are necessary to further characterize this.

Small pilot studies lend themselves to examination of individual treatment responses, particularly in cases such as this, in which the natural history of the disease is well studied and characterized. In a pilot study, the first objective is often to establish a likelihood of a biological effect beyond the chance of a type I error, ie, whether any individual experienced a significant treatment effect. As can be seen from the case report describing the response of patient S8, a significant treatment effect resulting from perispinal etanercept is the most likely explanation for the clinical response observed. The
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**Conclusion**

An increasing amount of basic science and clinical evidence implicates inflammatory processes and resulting glial
activation in the pathogenesis of AD. This small, open-label pilot study suggests that inhibition of the inflammatory cytokine TNF-alpha may hold promise as a potential approach to AD treatment. Further study in randomized, placebo-controlled clinical trials is merited.

Table 1. Patient Characteristics at Baseline, Before Etanercept Treatment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>76.7 ± 10.9</td>
<td>52-94</td>
</tr>
<tr>
<td>Female, % (n)</td>
<td>60% (9)</td>
<td>--</td>
</tr>
<tr>
<td>Duration of symptoms (mo)</td>
<td>43.1 ± 37.9</td>
<td>8-120</td>
</tr>
<tr>
<td>MMSE score (n = 15)</td>
<td>18.2 ± 8.8</td>
<td>0-29</td>
</tr>
<tr>
<td>ADAS-Cog score (n = 11)</td>
<td>20.8 ± 10.5</td>
<td>7.3-41</td>
</tr>
<tr>
<td>SIB score (n = 5)</td>
<td>62.5 ± 28.05</td>
<td>28-92</td>
</tr>
<tr>
<td>Prior Treatments:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memantine, % (n)</td>
<td>73% (11)</td>
<td>--</td>
</tr>
<tr>
<td>Duration before etanercept (mo)</td>
<td>10.6 ± 4.0</td>
<td>1.5-15</td>
</tr>
<tr>
<td>Donepezil, % (n)</td>
<td>47% (7)</td>
<td>--</td>
</tr>
<tr>
<td>Duration before etanercept (mo)</td>
<td>44.7 ± 47.9</td>
<td>10-120</td>
</tr>
<tr>
<td>Rivastigmine, % (n)</td>
<td>27% (4)</td>
<td>--</td>
</tr>
<tr>
<td>Duration before etanercept (mo)</td>
<td>5.6 ± 3.3</td>
<td>1-8</td>
</tr>
<tr>
<td>Galantamine, % (n)</td>
<td>13% (2)</td>
<td>--</td>
</tr>
<tr>
<td>Duration before etanercept (mo)</td>
<td>40.5 ± 6.4</td>
<td>36-45</td>
</tr>
<tr>
<td>Only 1 of the above, % (n)</td>
<td>40% (6)</td>
<td>--</td>
</tr>
<tr>
<td>Memantine + a cholinesterase inhibitor, % (n)</td>
<td>60% (9)</td>
<td>--</td>
</tr>
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</table>

MMSE = Mini-Mental State Examination; ADAS-Cog = Alzheimer’s Disease Assessment Scale-Cognitive subscale; SIB = Severe Impairment Battery

Table 2. Summary of Mixed Model Linear Regression Results Following Initiation of Perispinal Etanercept

<table>
<thead>
<tr>
<th>Measure (n)</th>
<th>Baseline Mean (SD)</th>
<th>Mean Change at 1 mo (SD)</th>
<th>Mean Change at 2 mo (SD)</th>
<th>Mean Change at 3 mo (SD)</th>
<th>Mean Change at 4 mo (SD)</th>
<th>Mean Change at 5 mo (SD)</th>
<th>Mean Change at 6 mo (SD)</th>
<th>Results of Regression Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE (15)</td>
<td>18.2(8.8)</td>
<td>-.29(1.82)</td>
<td>+1.07(2.01)</td>
<td>+1.87(1.99)</td>
<td>+2.00(2.13)</td>
<td>+1.93(2.34)</td>
<td>+2.13(2.23)</td>
<td>F (1,84) = 39.00, P &lt; .001</td>
</tr>
<tr>
<td>ADAS-Cog (11)</td>
<td>20.85 (10.5)</td>
<td>-4.28(3.44)</td>
<td>-4.64(4.36)</td>
<td>-4.67(5.97)</td>
<td>-7.14(4.51)</td>
<td>-4.52(4.80)</td>
<td>-5.48(5.08)</td>
<td>F (1,61) = 11.72, P &lt; .002</td>
</tr>
<tr>
<td>SIB (5)</td>
<td>62.5 (28.05)</td>
<td>+4.67(6.35)</td>
<td>+8.2(3.56)</td>
<td>+11.75(6.45)</td>
<td>+13.6(10.89)</td>
<td>+13.0(13.69)</td>
<td>+16.6(14.52)</td>
<td>F (1,26) = 22.60, P &lt; .001</td>
</tr>
</tbody>
</table>

MMSE = Mini-Mental State Examination; ADAS-Cog = Alzheimer’s Disease Assessment Scale-Cognitive subscale; SIB = Severe Impairment Battery
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Disclosure: Edward Tobinick, MD, has disclosed that he is the holder of patents, both issued and pending, including US patents 6,177,077, 6,419,934, 6,982,089, and others, which claim the use of TNF-alpha inhibitors, including etanercept, to treat Alzheimer's disease. Dr. Tobinick has also disclosed that he owns stock in Amgen, the manufacturer of etanercept, and had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

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Disclosure: Alan Weinberger, MD, has disclosed no relevant financial relationships.

Disclosure: Hart Cohen, MD, FRCPC, has disclosed no relevant financial relationships.