Effects of long-term etanercept treatment on anxiety- and depression-like neurobehaviors in rats

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HIGHLIGHTS
• Etanercept decreased anxiety-like neurobehaviors of rats.
• Etanercept decreased depressive-like neurobehaviors.
• Etanercept did not affect locomotor activity.
• These findings point to role for TNF-α in the modulation of emotional processes.

A B S T R A C T
Growing evidence indicates that there is a correlation between depression and inflammation. Administration of anti-tumor necrosis factor (TNF) agents for treatment of chronic inflammatory diseases, such as psoriasis, was associated with decreased depressive symptoms and increased quality of life in some clinical studies. The aim of the present study was to investigate the effects of chronic etanercept, a TNF-α inhibitor, on anxiety- and depression-like neurobehaviors in rats.

Male rats were treated for 8 weeks with either saline or etanercept (0.8 mg/kg/week, subcutaneously). The anxiety levels of rats were evaluated using the elevated plus maze, a classical rodent model of anxiety and depression was measured using the forced swimming test, a behavioral despair task.

The anxiety-like neurobehaviors of the animals were found significantly decreased after the etanercept treatment. Etanercept significantly decreased immobility time in rat model of despair test, seemed to have an antidepressive effect in rats. Compared to saline treatment, long-term etanercept treatment had no effect on the total number and pattern of locomotor activities.

Findings of the study supported the hypothesis that TNF-α has a role in the modulation of emotional processes and its inhibition may represent a novel strategy for the treatment of affective disorders.

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1. Introduction
A growing number of studies show high co-morbidity of anxiety and depression with chronic inflammatory diseases such as psoriasis and rheumatoid arthritis [1,2]. The relationship between depression and/or anxiety and chronic inflammatory diseases can be explained in several different manners. One explanation is that several circulating proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) are implicated in the origin of both conditions [3]. Moreover, it has been suggested that proinflammatory cytokines contribute to the development of depressive disorder and induce true major depressive disorders in physically ill patients with no history of mental disorders [4]. Data indicate that activation of the inflammatory response leads to release of inflammatory cytokines and mobilization of immune cells both of which have been shown to access the brain and alter behavior [5]. This is regarded as evidence that inflammation is an important biological event that can increase the risk of major depressive episodes, much like the more traditional psychosocial factors [6]. Consistently, administration of anti-TNF agents for the treatment of these chronic inflammatory diseases was associated with decreased depressive symptoms and increased health-related quality of life in addition to alleviation of disease symptoms [3,7–10]. Although these studies suggested that treatment with anti-TNF agents reduces symptoms of depression, it is not clear whether improvement in psychiatric pathology can be attributed

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to the primary effect of TNF–α inhibition or a consequence of clinical re-
mission. Moreover, our previous study showed that chronic treatment
with infliximab, a TNF–α inhibitor, significantly decreased the immobility
time in forced swim in chronically stressed rats, indicating a possible
antidepressant-like effect, as well as anxiolytic effect [11]. Therefore, we
decided to explore whether long-term etanercept treatment could affect
the anxiety- and depression-like neurobehaviors of rats without a chronic
inflammatory or stressful condition by various behavioral methods.

2. Materials and methods

2.1. Animals

Subjects were adult male Wistar albino rats weighing 300–400 g.
Animals were maintained under standard laboratory conditions on a
12/12-h light/dark cycle (lights on at 7:00 AM). Tap water and food
pellets were provided ad libitum. The experiments reported in this
study were conducted in accordance with the Regulation of Animal
Research Ethics Committee in Turkey (6 July 2006, Number 26220).
Ethical approval was granted by the Kocaeli University Animal Research
Ethics Committee (Kocaeli, Turkey).

All animals used in this study were naive to the experimental tests.
Rats were allocated to the following study groups: animals treated
with saline (control group, n = 9) and animals treated with etanercept
(etanercept group, n = 9). Etanercept (Wyeth, Münster, Germany)
dissolved in physiological saline and injected subcutaneously at a
dose of 0.8 mg/kg. Drug dosage was chosen according to the previous
clinical and experimental studies. Drug solutions were freshly pre-
pared prior to use once a week for 8 weeks since efficacy of classical
antidepressants appears after long term usage in forced swimming
test. Behavioral tests were started 3 days after the last injection
and performed on different days in the following order: locomotor
activity, the elevated plus maze and forced swimming. All experi-
ments were conducted between 09:00 AM and 12:00 PM.

2.2. Behavioral tests

2.2.1. Locomotor activity

Locomotor activity was assessed using an animal activity monitor-
ing system (Commat Ltd., Ankara, Turkey), which was composed of a
Plexiglas chamber, a computer, and open field activity software. The
Plexiglas chamber (42 × 42 × 30 cm) was equipped with infrared
photocells, and pairs of 15 infrared photobeams and detectors were
mounted horizontally every 2.5 cm (bottom) and vertically every
4.5 cm (upper). Interruptions of photocell beams were detected by
the computer system. Locomotor activity was recorded as stereotypic
(repeated beam breaks at the same photobeams), vertical (breaks in
the upper set of photobeams), and ambulatory (breaking more than
1 consecutive photobeam in the bottom set of photobeams) activities.
Total locomotor activity is expressed as the sum of stereotypic, ambu-
latory, and vertical activities. The activity was monitored continuously
for 10 min following acclimatization to the test room for a period of
1 h.

2.2.2. Elevated plus maze

Anxiety-like neurobehavior was evaluated in the elevated plus
maze apparatus, which was made of wood and consisted of 2 open
arms (50 × 10 cm) and 2 closed arms (50 × 10 cm) connected by a
central square (10 × 10 cm) and elevated to a height of 50 cm.
Rats were placed individually in the center of the maze facing a
closed arm and allowed 5 min of free exploration. The rat was con-
sidered to have entered an arm when all 4 limbs were inside the
arm. The apparatus was cleaned with ethanol solution after each
test. The percentage of time spent on the open arms and open arm
entries was calculated.

2.2.3. Forced swimming test

The forced swimming test apparatus was a cylinder (height, 47 cm;
inside diameter, 38 cm) containing 38 cm of tap water maintained at
22 ± 1 °C. The experimental session consisted of 2 trials: conditioning
and test. During the conditioning trial, rats were gently placed into the
cylinder for 15 min. After the trial, rats were dried and placed into a
warm cage with paper towels for 10–15 min before being returned to
their home cages. The test trial was carried out 24 h later. Rats were
placed again into the cylinder for a 5-min test session. Following the
swim session, rats were removed from the cylinder, dried with a towel,
and placed underneath a heating lamp for approximately 30 min before
being returned to their home cages. Tests were videotaped. The immo-
bility time, which was defined as the lack of motion of the whole body
except for the small movements necessary to keep the animal’s head
above the water, was recorded. The observer blind to the treatment condi-
tions recorded the time spent immobile in the test session.

2.3. Statistical analysis

Results are expressed as mean ± SEM. Student’s t test was used
for comparisons between groups. P values of <0.05 were considered
statistically significant.

3. Results

3.1. Effects of long-term etanercept treatment on locomotor activity

The locomotor activity of rats was measured in a 10-min trial
conducted on the third day of the last drug injection. Etanercept had
no effect on total locomotor activity [t(16) = 0.62; P = 0.54] or percent
of resting time [t(16) = 0.39; P = 0.69], indicating that the effects of
etanercept on emotional tests do not result from its effects on locomotor
activities (Table 1). Similar results were found when we analyzed further
components of the animals’ locomotor activity, including ambulatory
[t(16) = 0.72; P = 0.48], vertical [t(16) = 1.62; P = 0.12], and stereo-
typic [t(16) = 0.43; P = 0.67] activities (Table 1).

3.2. Effects of long-term etanercept treatment on anxiety-like neurobehavior
in the elevated plus maze test

Elevated plus maze tests were conducted on the fourth day of the last
drug injection. The etanercept-treated group spent a larger percentage
of time in the open arms [t(18) = 4.13; P < 0.001] (Fig. 1A) and exhibited a
higher proportion of open arm entries [t(18) = 2.34; P < 0.05] than the
control group did (Fig. 1B).

3.3. Effects of long-term etanercept treatment on depression-like
neurobehavior in the forced swimming test

Forced swimming test was conducted for the assessment of depressive-like behavior (despair) on the 5th and 6th days of the last
drug injection. On the first day of forced-swim testing, the immobility
time of etanercept-treated rats did not differ from that of the controls
[t(16) = 1.43; P = 0.17] (Fig. 2). However, etanercept-treated rats
exhibited significantly less immobility than that of the controls on the
second day of testing [t(16) = 5.24; P < 0.0001] (Fig. 2).

4. Discussion

Long-term etanercept treatment significantly decreased anxiety-
and depressive-like neurobehaviors of rats as assessed with elevated
plus maze and forced swimming tests, respectively. Etanercept did not
alter total locomotor activity levels, which indicated that differences
observed in emotional tests were not mediated by the psychomotor
activation of etanercept.
Recent neurobiological studies have revealed that cytokines are involved in the development and maintenance of mood disorders. According to the so-called ‘cytokine hypothesis of depression’, cytokines represent the key factors in the central mediation of the behavioral, neuroendocrine, and neurochemical features of depressive disorders [12,13]. Of the major proinflammatory cytokines, TNF-α is particularly interesting with respect to major depression. Both TNF-α and its receptor levels are significantly elevated in acutely depressed patients [4], and improvement of depression correlates with decreases in serum levels of TNF-α [14]. Preclinical studies also support these findings. In animals, it was shown that anxiety and/or depressive-like behaviors were induced by both TNF-α and inducers such as lipopolysaccharide administration or chronic mild stress (CMS) [15–21]. Consistently, antidepressant treatment has been shown to decrease TNF-α level both in humans and animals [22–24]. All these evidence suggest that there is an association between pro-inflammatory cytokines and depression but the mechanisms by which inflammation affects mood are only partially understood.

In recent years, several studies have suggested that treatment with anti-TNF agents for psoriasis also reduces symptoms of depression in these patients [3,7–9]. First, a prospective clinical study reported by Tyring and co-workers [7] demonstrated that compared to placebo administration, etanercept treatment led to an improvement of at least 50% in depression rating scales at week 12. However, the improvement in symptoms of depression was not strongly correlated with the improvement in psoriasis area and severity index (PASI) in this study [7].

Subsequently, Bassukas and co-workers [9] reported that treatment with infliximab resulted in stabilization or improvement of the manifestations of psychiatric morbidity in 3 psoriasis patients with overt psychiatric disorders, including recurrent depression and bipolar disorder. Finally, Menter et al. [3] showed that adalimumab treatment reduced symptoms of depression and improved health-related quality of life in addition to improving psoriasis. In contrast to Tyring et al. [7], they reported that reductions in depression symptoms were correlated with PASI [3]. Although these studies have suggested that treatment with anti-TNF agents for psoriasis reduces symptoms of depression, none of these studies could distinguish whether anti-TNF agents had a direct or indirect effect (a secondary effect representing alleviation of psoriasis) on depression. Several animal studies have shown that cytokine-induced sickness behaviors, which are similar to depressive-like behaviors, can be attenuated by TNF-α blockade [16,17]. Consistent with clinical observations that point at the antidepressant efficacy of TNF alpha inhibitors, we reported that chronic administration of infliximab decreased CMS-induced depression-like behaviors in rats [11].

The common outcome of all these studies is that, inflammation could lead to depression symptoms and TNF-α inhibition could display antidepressant/anxiolytic action in chronic inflammatory diseases. On the other hand, the effects of TNF-α inhibition on the anxiety and depression independently of chronic inflammation have not been evaluated. In order to clarify this topic, we used depression and anxiety tests which are commonly used in preclinical investigation, in the naive rats. In this context, our results, that showed a reduction in anxiety and depressive-like symptoms after chronic etanercept treatment are novel and might have a base for future studies for investigation of underlying mechanisms of the antidepressant and anxiolytic effects of anti-TNF agents.

In order to clarify the anti-depressive effects of TNF-α inhibitors, several mechanisms could be speculated based on previous studies: (1) TNF-α might acutely regulate neuronal serotonin transporter activity [7,25]. (2) Immune activation with increased production of pro-inflammatory cytokines activates the tryptophan and serotonin-degrading enzyme indoleamine-2,3-dioxygenase. (3) TNF-α might play a causative role in depression-related activation of the hypothalamus–pituitary–adrenal axis [26]. Therefore, etanercept as a TNF-α inhibitor, might block the depressive and anxiety-like behaviors by interacting with one or more plausible peripheral mechanisms maintained above and others such as modulation of hypothalamic pituitary adrenal axis and vagal activity [27,28].

The antidepressant and anxiolytic effects of etanercept could be attributed to either its chronic effects on the baseline circumstances modulating the emotional and behavioral responses to acute stressors or its acute effects on possibly higher TNF-α levels after the acute stress. Although, elevated plus maze and forced swimming tests are acute models of the anxiety and depression, efficacy of classical antidepressants usually requires long-term treatment in the forced swimming test indicating that antidepressant actions of these drugs are due to neuroplastic changes capable of modifying the stress-induced response [29–31]. Based on this evidence, we can also argue that the action pattern of etanercept may resemble those of antidepressant drugs (especially selective serotonin reuptake inhibitors). As a second possibility, etanercept could inhibit TNF-α induced by procedures applied during behavioral tests. Although, CMS that is used to establish

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**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Stereotypic activity</th>
<th>Vertical activity</th>
<th>Ambulatory activity</th>
<th>Total activity</th>
<th>% resting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>592.8 ± 48.40</td>
<td>15.13 ± 1.576</td>
<td>39.63 ± 17.71</td>
<td>648.1 ± 56.56</td>
<td>73.0 ± 2.24</td>
</tr>
<tr>
<td>Etanercept</td>
<td>618.3 ± 33.82</td>
<td>20.25 ± 2.732</td>
<td>57.88 ± 18.13</td>
<td>696.4 ± 52.59</td>
<td>71.5 ± 3.08</td>
</tr>
</tbody>
</table>

There was no difference between the groups in total activity and percentage of resting time compared with saline as well as in stereotypic, ambulatory, or vertical activity (all P > 0.5). Values depict mean ± SEM.

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**Fig. 1.** Effects of chronic etanercept treatments on the anxiety-like behavior in elevated plus maze. Etanercept treated group spent more time (percentage) in the open arms and exhibited higher proportion of open arm entries. * depicts P < 0.05, *** depicts P < 0.001. Values depict mean ± SEM.
Depression has been shown to increase TNF-α levels [20,21], to our knowledge there is no information regarding the effects of elevated plus maze or forced swimming tests on TNF-α levels. The measurement of TNF-α level after these tests is needed.

5. Conclusion

Our results showed that etanercept treatment reduced the anxiety and depressive-like symptoms of rats in the absence of a chronic inflammatory or stressful condition, which is consistent with the clinical studies mentioned above. This indicates that new indications for TNF-α blockers will emerge in the future. The acute effects of these agents on emotional symptoms and the underlying mechanisms remain to be investigated.

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References


