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Nitric oxide modulation mediates the protective effect of trazodone in a mouse model of chronic fatigue syndrome

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Abstract:
The present study was conducted with the aim of elucidating the possible role of nitric oxide (NO) in the neuroprotective effects of trazodone used to treat chronic fatigue syndrome (CFS) in mice. Male albino mice were forced to swim for a six minute session each day for 7 days and the immobility period was recorded every other day. Trazodone (5 mg/kg and 10 mg/kg) was administered each day 30 min before the forced swim test. In addition, L-arginine (100 mg/kg) and L-NAME (5 mg/kg) were administered 15 min before administration of trazodone (5 mg/kg). Various behavioral tests, including locomotor (actophotometer) and anxiety (mirror chamber and plus maze) tests, as well as biochemical parameters (lipid peroxidation, reduced glutathione, catalase, and nitrites) were evaluated on the 8th day. Forced swimming for 7 days caused a chronic fatigue-like condition, anxiety-like behavior, impairments in locomotor activity, and oxidative damage (increased lipid peroxidation and nitrite levels, and depletions in the reduced forms of glutathione and catalase activity) in animals. Pretreatment with L-NAME (5 mg/kg) potentiated the antioxidant effect of trazodone (5 mg/kg). However, L-arginine (100 mg/kg) pretreatment reversed the protective effect of trazodone (5 mg/kg) ($p < 0.05$). The present study suggests the possible involvement of NO signaling in the protective effect of trazodone.

Key words:
anxiety, locomotor activity, oxidative stress, chronic fatigue syndrome, trazodone, L-NAME, L-arginine

Abbreviations: CFS – chronic fatigue syndrome, DTNB – dithiobisnitrobenzoic acid, H_2O_2 – hydrogen peroxide, MDA – malondialdehyde, NO – nitric oxide, ROS – reactive oxygen species

Introduction

Chronic fatigue syndrome (CFS) is a common illness characterized by persistent and relapsing fatigue, specifically affecting 522 women and 291 men per 100,000 [9]. CFS patients often complain of headache, joint pain, gastrointestinal disturbance, cognitive dysfunction, visual disturbance, paresthesia, and neuropsychiatric problems [12, 13]. Because there is no known cause of CFS, current treatments remain symptom-based, with a focus on patient management rather than a cure. Various therapies, such as cognitive behavior therapy, graded exercise therapy, pharmacological interventions (e.g. antidepressants and corticosteroids), and nutritional supplements (antioxidants), have been used with limited success [35]. Although these drugs reduce the level of fatigue in patients [38], the exact mechanism or scientific explana-

tion for how these therapies reduce fatigue and other related problems in these CFS patients have yet to be understood.

Recent studies have demonstrated that oxidative stress is involved in the pathophysiology of CFS, which significantly contributes to its pathology and the appearance of clinical symptoms [5, 36]. The oxidative stress mechanisms of CFS need to be understood in order to design therapeutic strategies. Short-term stressors capable of increasing nitric oxide (NO) levels can initiate episodes of illness in multiple disease states including chronic fatigue syndrome, multiple chemical sensitivity, fibromyalgia, and post-traumatic stress disorder [28]. These stressors, acting primarily through NO products (such as peroxynitrites), are thought to initiate a complex pathological cycle, which is responsible for chronic illness. The intricacies of the NO/ONOO- cycle reflect the complex mechanisms contributing to disease pathologies involving oxidative stress.

Oxidative stress and NO have been proposed to play an important role in CFS pathophysiology [24]. However, the exact cellular cascades are not fully understood thus far.

Tradozone has been successfully used to treat depression, however, the exact mechanisms by which it alleviates depressive like symptoms in CFS patients are unknown [22]. Trazodone is a serotonin reuptake inhibitor and antagonist of 5-hydroxytryptamine (5-HT) receptors. It was previously found that the N-methyl-D-aspartate (NMDA) receptor/NO/cyclic GMP pathway can be inhibited by 5-HT acting at receptors of the 5-HT_{2C} type [23]. In addition, a recent study has also shown that inhibition of NO synthase could be used to enhance the clinical efficacy of serotonergic antidepressants [8].

Therefore, the main objective of this study was to explore the possible role of NO in the protective effect of trazodone in an animal model of CFS.

Materials and Methods

Animals

Male laca mice (20–25 g) bred in the central Animal House of Panjab University were used in the study. The animals were housed under standard laboratory conditions and were supplied with food and water *ad libitum*. The animals were maintained on a 12-h light/dark cycle and were acclimatized to the laboratory conditions prior to experimentation. All of the experiments were carried out between 10:00 and 17:00 h. The animals were drawn at random for the study. The experimental protocol was approved by the Institutional Animal Ethics Committee and was conducted according to the Indian Science Academy Guidelines for the use and care of experimental animals.

Drugs and treatment schedule

The animals were divided into different groups, consisting of six animals in each group. Trazodone (5 mg/kg and 10 mg/kg) was administered intraperitoneally (*ip*) daily, 30 min before the forced swim test. L-arginine (100 mg/kg) and L-NAME (5 mg/kg) were administered (*ip*), 15 min before trazodone treatment.

Forced swim test (measurement for period of immobility)

The animals were forced to swim individually in a glass jar (25 × 12 × 25 cm) containing water at room temperature (22 ± 3°C). The water depth was adjusted to 15 cm and was kept constant throughout the experiments. After an initial period of vigorous activity, each animal assumed a typical immobile posture. The duration of immobility was measured during a total period of 6 min. The mice were judged to be immobile when they ceased any struggling movements of their limbs to keep their head above water. They were forced to swim for a six minute session each day for seven days. The prolongation of the immobility period is considered a situation similar to CFS [11].

Behavioral assessment

The following behavioral tests were conducted 24 h after the last forced swim test.

Elevated plus maze test

The elevated plus maze [16], is a test for assessing anxiogenic and anxiolytic drug effects in rodents. The plus maze apparatus consists of two open (16 × 5 cm) and two closed arms (16 × 5 × 12 cm), placed at a height of 25 cm above the floor. Mice were placed

individually at the center of the elevated plus maze with their head facing toward an open arm. During the 5 min test, the average time spent (duration in open arm/number of entries in open arm) in the open arms of the maze was recorded.

Mirror chamber test

The mirror chamber consisted of a wooden chamber having a mirror chamber enclosed within it. During the 5 min test session, the following parameters were noted: a) latency to enter the mirror chamber, b) average time spent in the mirror chamber. Animals were placed individually at the edge of the distal corner of mirror chamber at the beginning of the test. An anxiogenic response is defined as a decrease in the average time spent in the mirror chamber [16].

Measurement of ambulatory activity

The ambulatory activity was recorded using an actophotometer (IMCORP, Ambala, India). Before the actual recording of locomotor activity, each animal was placed individually in the activity meter for 3 min as a habituation period. The locomotor activity was recorded using the actophotometer for a period of 5 min. Ambulatory activity was recorded and expressed in terms of total photo beam counts for 5 min per animal [15].

Biochemical tests

Dissection and homogenization

On day 8, after behavioral quantification, animals were sacrificed by decapitation. The whole brains were removed and a 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). Homogenates were centrifuged for 20 min at 15,000 g and the supernatant was used for the estimation of lipid peroxidation and reduced glutathione levels. The postnuclear fractions for the catalase assay were obtained by centrifugation of the homogenate at 1,000 g for 20 min at 4°C. For the other enzyme assays, the homogenate was centrifuged at 12,000 g for 60 min at 4°C.

Lipid peroxidation assay

The quantitative measurement of lipid peroxidation was performed as described by Wills [41]. The amount of malondialdehyde (MDA), a measure of lipid peroxidation, was measured by a reaction with thiobarbituric acid (532 nm) with a Shimadzu spectrophotometer (Kyoto, Japan). The values were calculated using the molar extinction coefficient of the chromophore (1.56×10^5 $M^{-1}cm^{-1}$) and were expressed as a percentage of the control.

Estimation of reduced glutathione

Reduced glutathione in the brain was estimated according to the method described by Ellman [4]. Supernatant (1 ml) was precipitated with 1 ml of 4% sulfosalicylic acid and digested at 4°C for 1 h. The sample was centrifuged at 1,200 g for 15 min at 4°C. To 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were added. As the yellow color developed, it was read immediately at 412 nm using a Shimadzu spectrophotometer. Results were calculated using the molar extinction coefficient of the chromophore (1.36×10^4 $M^{-1}cm^{-1}$) and were expressed as a percentage of the control.

Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of NO, was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) as described by Green et al. [7]. Equal volumes of the supernatant and Greiss reagent were mixed, and the mixture was incubated for 10 min at room temperature and the absorbance was measured at 540 nm using a Shimadzu spectrophotometer. The concentration of nitrite in the supernatant was determined from a standard curve and was expressed as a percentage of the control.

Protein estimation

The protein content was measured according to the method of Lowry et al. [19] using bovine serum albumin as a standard.

Estimation of catalase

Catalase activity was assayed by the method of Luck et al. [20], wherein the breakdown of H_2O_2 was measured at 240 nm. Briefly, the assay mixture consisted

of 3 ml of H_2O_2 phosphate buffer (1.25×10^{-2} M H_2O_2) and 0.05 ml of the brain homogenate supernatant (10%). The change in absorbance was recorded at 240 nm using a Shimadzu spectrophotometer. Enzyme activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.07). The result was expressed as micromoles of H_2O_2 decomposed per min/mg protein.

Statistical analysis

All the values are expressed as the mean ± SEM. The data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's test. For all analyses, the test criterion for statistical significance was $p < 0.05$.

Results

Effect of trazodone and its modulation by L-NAME or L-arginine on mean immobility period

Daily six minute sessions of forced swimming significantly increased the immobility period on the 3rd, 5th, and 7th day as compared to a naive animal ($p < 0.05$). Pretreatment with trazodone (5 mg/kg and 10 mg/kg) dose dependently reduced the immobility period as compared to control (chronic fatigue) ($p < 0.05$) (Tab. 1). L-arginine, a NO precursor, at a dose of 100 mg/kg did not significantly influence the immobility period as compared to control ($p < 0.05$). However, pretreatment with L-arginine (100 mg/kg) significantly reversed the protective effect of trazodone (5 mg/kg) ($p < 0.05$). L-NAME, a nitric oxide synthase inhibitor, at a dose of 5 mg/kg significantly reduced the immobility period for each day examined as compared to the control ($p < 0.05$) (Tab. 1). Furthermore, L-NAME pretreatment combined with trazodone caused a further reduction in the immobility period when compared to the effects of either drug alone ($p < 0.05$) (Tab. 1).

Effect of trazodone and its modulation by L-NAME or L-arginine on ambulatory activity

Daily six minute sessions of forced swimming for seven days significantly impaired locomotor activity as compared to the naive group (without chronic fatigue) ($p < 0.05$). Trazodone (5 mg/kg and 10 mg/kg) pretreatment significantly reversed the reduced locomotor activity compared to control conditions (chronic fatigue) ($p < 0.05$) (Fig. 1). L-arginine at a dose of 100 mg/kg did not significantly influence locomotor activity as compared to the control ($p < 0.05$). Furthermore, pretreatment with L-arginine (100 mg/kg) did not attenuate the protective effect of trazodone (5 mg/kg) (Fig. 1).

Tab. 1. Effect of trazodone and its modulation by L-arginine or L-NAME on the immobility period

Drug treatment (mg/kg)	Immobility period (s) after			
	1st day	3rd day	5th day	7th day
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Naive	175.6 ± 2.4	176.2 ± 2.3	176.0 ± 3.0	172 ± 4.0
Control (chronic fatigue)	179.7 ± 7.40	201.7 ± 7.18[a]	258.6 ± 4.79[a]	282.4 ± 5.09[a]
TRD (5)	124.25 ± 5.25[b]	133.5 ± 6.1[b]	140 ± 5.95[b]	148.25 ± 7.2[b]
TRD (10)	110.5 ± 8.9[b,c]	119 ± 7.8[b,c]	127.25 ± 6.5[b,c]	128.75 ± 6.8[b,c]
L-ARG (100)	173.5 ± 9.8	193.9 ± 8.6	243.75 ± 7.9	269.0 ± 8.3
L-NAME (5)	134.8 ± 5.4[b]	153.8 ± 6.3[b]	161.6 ± 5.9[b]	170.6 ± 7.8[b]
L-ARG (100) + TRD (5)	149.5 ± 10.8[c]	157.5 ± 9.4[c]	172.75 ± 7.9[c]	186 ± 8.3[c]
L-NAME (5) +TRD (5)	78.2 ± 11.2[c,d]	96.2 ± 9.4[c,d]	112.2 ± 10.4[c,d]	115.7 ± 6.3[c,d]

Values are the mean ± SEM; [a] $p < 0.05$ as compared to naive (non-fatigue), [b] $p < 0.05$ as compared to control (chronic fatigue), [c] $p < 0.05$ as compared to trazodone (5 mg/kg), [d] $p < 0.05$ as compared to L-NAME (5 mg/kg) (repeated measures one-way ANOVA followed by Tukey's test). TRD – trazodone, L-ARG – L-arginine

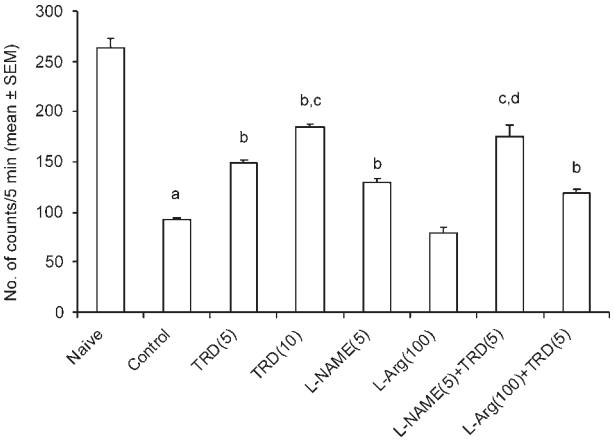

Fig. 1. Effect of trazodone and its modulation by L-arginine and L-NAME on locomotor activity. Values are the mean ± SEM; [a] $p < 0.05$ as compared to naive (non-fatigue), [b] $p < 0.05$ as compared to control (chronic fatigue), [c] $p < 0.05$ as compared to trazodone (5 mg/kg), [d] $p < 0.05$ as compared to L-NAME (5 mg/kg) (repeated measures one-way ANOVA followed by Tukey's test). TRD – trazodone, L-ARG – L-arginine

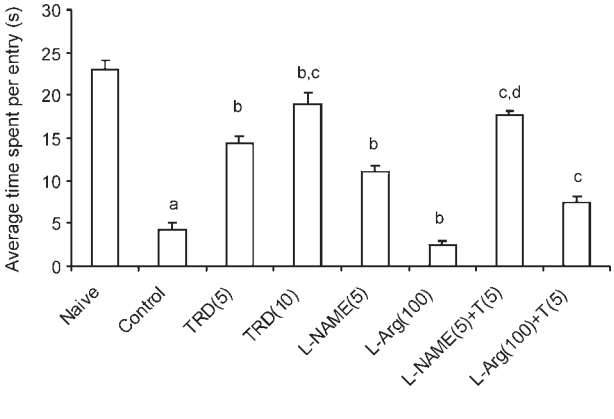

Fig. 2. Effect of trazodone and its modulation by L-arginine and L-NAME on the plus maze test. Values are the mean ± SEM; [a] $p < 0.05$ as compared to naive (non-fatigue), [b] $p < 0.05$ as compared to control (chronic fatigue), [c] $p < 0.05$ as compared to trazodone (5 mg/kg), [d] $p < 0.05$ as compared to L-NAME (5 mg/kg) (repeated measures one-way ANOVA followed by Tukey's test). TRD – trazodone, L-ARG – L-arginine

L-NAME at a dose of 5 mg/kg, significantly improved locomotor activity compared to the control ($p < 0.05$) (Fig. 1). Furthermore, L-NAME (5 mg/kg) pretreatment with trazodone (5 mg/kg) caused further improvements in locomotor activity, which was significant compared to their effects alone ($p < 0.05$) (Fig. 1).

Effect trazodone and its modulation by L-NAME or L-arginine on anxiety (plus maze and mirror chamber test)

Seven days of forced swimming significantly caused anxiolytic-like activity in both the plus maze (decreased average time spent per entry in an open arm) and the mirror chamber test (decreased latency to enter and average time spent per entry in the mirror chamber), which was significant compared to the naive group (without chronic fatigue) ($p < 0.05$) (Fig. 2 and Tab. 2). Trazodone (5 mg/kg and 10 mg/kg) pretreatment (once daily for seven days) significantly caused anxiolytic-like effects in both test models (i.e. increased average time spent per entry in an open arm in the case of the plus maze, and increased latency to enter and increased average time spent per entry in the mirror chamber), which was significantly different from the control (chronic fatigue) ($p < 0.05$) (Fig. 1). L-arginine (100 mg/kg) produced significant anxiety-like behaviors in both the test models compared to the control ($p < 0.05$) (Fig. 2 and Tab. 2). Furthermore, pretreatment with L-arginine (100 mg/kg) reversed the antianxiety effect of trazodone (5 mg/kg) in both test models compared to the effects of each drug alone ($p < 0.05$).

Tab. 2. Effect of trazodone and its modulation by L-arginine and L-NAME on the mirror chamber test

Drug treatment (mg/kg)	Latency to enter mirror chamber (s)	Average time spent per entry in mirror chamber (s)
	Mean ± SEM	Mean ± SEM
Naive	73 ± 3.01	21 ± 0.95
Control	139.2 ± 8.56[a]	7.25 ± 0.73[a]
TRD (5)	114 ± 1.05[b]	9.8 ± 0.37[b]
TRD (10)	108 ± 0.83[b]	13.4 ± 0.53[b]
L-NAME (5)	123 ± 2.68[b]	8.5 ± 0.94[b]
L-ARG (100)	167 ± 6.37	6.6 ± 0.49
L-NAME (5) + TRD (5)	94 ± 2.02[c,d]	10.3 ± 0.41[c,d]
L-ARG (100) + TRD (5)	145 ± 5.66[b]	7.3 ± 0.63[b]

Values are the mean ± SEM; [a] $p < 0.05$ as compared to naive (non-fatigue), [b] $p < 0.05$ as compared to control (chronic fatigue), [c] $p < 0.05$ as compared to trazodone (5 mg/kg), [d] $p < 0.05$ as compared to L-NAME (5 mg/kg) (repeated measures one-way ANOVA followed by Tukey's test). TRD – trazodone, L-ARG – L-arginine

Tab. 3. Effect of trazodone and its modulation by L-arginine and L-NAME on oxidative damage

Drug treatment (mg/kg)	LPO (nM of MDA/mgpr) (% of control)	Nitrite (μg/ml) (% of control)	Catalase (μM of H_2O_2/min/mgpr) (% of control)	GSH (nM of GSH/mg of protein) (% of control)
Naive	0.163 ± 0.003 (100%)	123 ± 5.96 (100%)	2.43 ± 0.091 (100%)	0.0321 ± 0.002 (100%)
Control	0.424 ± 0.005[a] (260.12%)	520 ± 27.2[a] (422.76%)	0.86 ± 0.075[a] (35.39%)	0.005 ± 0.001[a] (15.57%)
TRD (5)	0.315 ± 0.005[b] (193.25%)	302 ± 3.71[b] (245.52%)	1.23 ± 0.0063[b] (50.61%)	0.0143 ± 0.0009[b] (44.54%)
TRD (10)	0.237 ± 0.008[b,c] (145.39%)	150 ± 3.14[b,c] (121.95%)	2.06 ± 0.056[b,c] (84.77%)	0.028 ± 0.003[b,c] (87.22%)
L-NAME (5)	0.379 ± 0.002[b] (232.51%)	384 ± 12.34[b] (312.19%)	0.98 ± 0.045[b] (40.32%)	0.0149 ± 0.0008[b] (46.41%)
L-ARG (100)	0.482 ± 0.01 (295.7%)	573.3 ± 17.92 (466.09%)	0.66 ± 0.049 (27.16%)	0.003 ± 0.0005 (9.34%)
L-NAME (5) + TRD (5)	0.228 ± 0.005[c,d] (139.87%)	268 ± 15.86[c,d] (217.88%)	1.87 ± 0.0317[c,d] (76.95%)	0.039 ± 0.004 (121.49%)
L-ARG (100) + TRD (5)	0.303 ± 0.015[c] (185.88%)	410 ± 27.33[c] (333.33%)	1.02 ± 0.0493[c] (41.97%)	0.014 ± 0.002[c] (43.61%)

Values are the mean ± SEM; [a] $p < 0.05$ as compared to naive (non-fatigue), [b] $p < 0.05$ as compared to control (chronic fatigue), [c] $p < 0.05$ as compared to trazodone (5 mg/kg), [d] $p < 0.05$ as compared to L-NAME (5 mg/kg) (repeated measures one-way ANOVA followed by Tukey's test). TRD – trazodone, L-ARG – L-arginine

L-NAME (5 mg/kg) produced an anxiolytic-like effect in both test models (Fig. 2 and Tab. 2). However, pretreatment with trazodone (5 mg/kg) before L-NAME caused a dramatic improvement in the anti-anxiety effect, which was significant compared to the effects of either drug alone ($p < 0.05$) (Fig. 2 and Tab. 2).

Effect of trazodone and its modulation by L-arginine and L-NAME on lipid peroxidation, reduced glutathione, and the nitrite and catalase activity in the chronic fatigued brain

Seven days of forced swimming significantly raised lipid peroxidation, nitrite levels, and depleted reduced forms of glutathione and catalase activity as compared to naive animals (non-fatigue) ($p < 0.05$). Trazodone (5 mg/kg and 10 mg/kg) pretreatment dose dependently attenuated lipid peroxidation, nitrite activity, restored reduced forms of glutathione and catalase activity as compared to the control (chronic fatigue) ($p < 0.05$) (Fig. 1). L-arginine at a dose of 100 mg/kg caused oxidative damage as indicated by increased lipid peroxidation, nitrite levels, and depleted reduced forms of glutathione and catalase activity. Furthermore, pretreatment of L-arginine (100 mg/kg) with trazodone (5 mg/kg) reversed the antioxidant activity of trazodone (5 mg/kg) ($p < 0.05$) (Tab. 3). L-NAME at a dose of 5 mg/kg significantly improved the attenuation of oxidative damage ($p < 0.05$) (Tab. 3). In addition, L-NAME pretreatment with trazodone (5 mg/kg) caused further improvements in the antioxidant activity compared to L-NAME alone ($p < 0.05$) (Tab. 3).

Discussion

Chronic fatigue syndrome (CFS) is characterized by profound fatigue, infections, and rheumatological and neuropsychiatric symptoms. Recently, several hypotheses have been proposed to elucidate the pathophysiologies of CFS [32]. However, there is some evidence that CFS is accompanied by increased oxida-

tive stress and that NO pathways are involved in its pathogenesis [6, 21, 39]. Moreover, free-radical damage by reactive oxygen species (ROS) has been suggested to play a critical role in the pathophysiology of CFS and stress-induced depression [2, 10, 33].

Consistent with other published observations [11, 35], 7 days of forced swimming produced CFS-like symptoms indicated by increases in despair behavior (increased immobility period), anxiety-like behaviors, and reduced locomotor activity. These behavioral changes might be due to cumulative stress and chronic stress has been well documented to cause anxiety-like behaviors [11], reduce locomotor activity, and lead to stress-induced depression [11]. However, the mechanisms for these changes are not well understood. In addition, 7 days of forced swimming significantly raised lipid peroxidation, nitrite concentrations, and depleted the reduced forms of glutathione and catalase activity, thereby indicating oxidative damage. The role of oxidative stress and related free radical generation has been well documented in the pathogenesis of CFS [31, 32]. ROS generated by a severe stressor significantly compromise *in vivo* antioxidant defenses of animals submitted to CFS [25]. Accordingly, chronic oxidative damage alters mitochondrial function, regulation of calcium homeostasis [1], energy pathways [29], neuronal precursors, processes of neurogenesis [14], and cell death [3].

In the present study, trazodone pretreatment provided a protective effect by attenuating the behavioral and biochemical alterations induced by 7 days of forced swimming. Both the plus maze and the mirror chamber are well known tests for assessing the anxiolytic effect of drugs. In the present study, trazodone pretreatment improved locomotor activity, lead to anti-anxiety-like effects, and reduced the immobility period. Furthermore, trazodone pretreatment improved the anti-anxiety-like effects of L-NAME as indicated by the number of entries and the duration of time spent in the open arm of the elevated-plus maze, as well as significantly increasing the time spent in the mirror chamber. Clinical studies have also shown that antidepressant treatments, particularly SSRIs, positively influence the symptoms of CFS patients and reduce associated neuropsychiatry problems [34, 38]. However, the exact mechanisms of their beneficial effect are not clear. Therefore, this study attempted to elucidate the possible mechanisms underlying the behavioral and biochemical effects of trazodone treatment for CFS.

Seven days of trazodone pretreatment attenuated lipid peroxidation, reduced nitrite concentrations, and restored reduced glutathione levels and catalase activity. These findings suggest that trazodone has a direct ability to protect against highly damaging hydroxyl radicals that oxidize most cellular targets including lipids, proteins, and DNA [26, 40]. Interestingly, lipid peroxidation has been demonstrated to occur in the brain after the initial formation of large amounts of ROS during stress [18, 25]. Our study showed that treatment with trazodone effectively prevented membrane lipid peroxidation. Since antidepressants act on many different neurotransmitter systems and receptors, a common mechanism of action of antidepressants has eluded researchers for years. However, it has been proposed that one of the shared mechanisms of action of antidepressants is the upregulation of antioxidant enzymes [17, 25]. Our study provides evidence that trazodone enhanced antioxidant enzyme activity following chronic forced swimming exposure. In addition, chronic antidepressant treatment has been demonstrated to upregulate cAMP-response element-mediated gene expression in the rat cortex and hippocampus [37].

In the present study, L-arginine, a NO precursor, significantly increased anxiety-like behaviors (increased average time spent in both the mirror chamber and the plus maze test) and oxidative damage as indicated by increased lipid peroxidation and nitrite activity, thereby suggesting that free radical generation might contributed to the severity of these behavioral and biochemical alterations. L-arginine produces NO by acting on nitric oxide synthase, which combines with superoxide to form the potent oxidative agent, peroxynitrite. The ROS cycle eventually allows peroxynitrite to target the mitochondria and this may be related to observed mitochondrial dysfunction in CFS patients [27]. Thus, it seems that NO pathways could be involved in explaining the oxidative stress theory of chronic fatigue syndrome. However, L-NAME, a NO synthase inhibitor, protected against chronic forced swimming-induced behavioral and biochemical alterations. This finding further confirms the possible involvement of the NO pathway in the pathogenesis of CFS. In addition, pretreatment of L-arginine (a NO precursor) with trazodone caused a reversal of the neuroprotective effects. Moreover, L-NAME potentiated the neuroprotective effect of trazodone, thereby confirming the involvement of the nitric oxide pathway in the neuroprotective action of trazodone. In addition, trazodone has been reported to in-

hibit the NMDA receptor/NO/cyclic GMP system through activation of 5-HT_{2C} receptor subtypes [22]. Further studies may indicate that trazodone produces its neuroprotective action by altering the NO pathway. However, the exact cellular cascade responsible for the actions of trazodone is far from clear.

In conclusion, treatment with trazodone ameliorates forced swimming-induced behavioral alterations and oxidative damage. Therefore, our study suggests that enhancement of *in vivo* antioxidant defenses and improvements in the cellular antioxidant status might be an important mechanism underlying the neuroprotective action of trazodone. Thus, the present study indicates that the neuroprotective potential of trazodone involves the NO pathway.

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