Effects of fluoxetine, tianeptine and olanzapine on unpredictable chronic mild stress-induced depression-like behavior in mice

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A B S T R A C T

Aims: Tianeptine is an atypical antidepressant drug that has a different mechanism of action than other antidepressants. Olanzapine is an atypical antipsychotic drug used for the treatment of schizophrenia. The present study was undertaken to investigate effects of chronic administration of tianeptine or olanzapine on unpredictable chronic mild stress (UCMS)-induced depression-like behavior in mice compared to a widely used SSRI antidepressant, fluoxetine.

Main methods: Male inbred BALB/c mice were subjected to different kinds of stressors several times a day for 7 weeks and were treated intraperitoneally with tianeptine (5 mg/kg), olanzapine (2.5 mg/kg), fluoxetine (15 mg/kg) or vehicle for 5 weeks (n=7–8 per group).

Key findings: All the drugs tested prevented stress-induced deficit in coat state during UCMS procedure, in grooming behavior in the splash test, decreased the attack frequency in the resident intruder test and decreased the immobility time in the tail suspension test. In the open field test olanzapine had anxiolytic-like effects in both stressed and non-stressed mice. Tianeptine, olanzapine and fluoxetine decreased the enhanced levels of plasma ACTH and IL-6. Chronic treatment with tianeptine resulted in a significant increase in both total number and density of BrdU-labeled cells in stressed animals, while fluoxetine and olanzapine had a partial effect.

Significance: The results of this study support the hypothesis that tianeptine can be as effective as fluoxetine for the treatment of depression in spite of the differences in the mechanism of action of these drugs. Moreover, olanzapine could be used effectively in psychotic patients with depression.

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Introduction

Depression is a serious emotional disorder with high morbidity and mortality (Kiecolt-Glaser and Glaser, 2002). It is caused by several stress factors and leads to changes in neurotransmitter levels in the brain, important changes in neuronal dendrite structure and function and histopathological changes in the volume and structure of important brain areas.

The atypical antidepressant tianeptine, which has a mechanism of action opposite that of SSRIs, exerts its antidepressant activity by increasing serotonin reuptake (Mennini et al., 1987). The commonly used atypical antipsychotic olanzapine, which is proposed to have antidepressant-like activity, exerts its effect via both serotoninergic and dopaminergic receptors (Marston et al., 2011; NemeroFF, 2007).

Tianeptine, similar to fluoxetine, diminishes the neuronal degeneration caused by depression. Tianeptin reverses the effects of chronic stress on brain plasticity in animal depression models (Czéh et al., 2001; Vouimba et al., 2003). Fluoxetine is a selective serotonin (5-HT) reuptake inhibitor, and its antidepressant features are well known (Mutlu et al., 2009).

Chronic therapy with both antidepressants and antipsychotics decreases the anhedonic symptoms observed in schizophrenic and depressive patients, reverses cognitive disturbances and increases
neurogenesis (Manji et al., 2001; Marston et al., 2011; McEwen and Olie, 2005). Moreover, important alterations in neurotransmitter systems have been detected in depression and schizophrenia. In animals exposed to long-term stress, BDNF (brain-derived neurotrophic factor), an important neurotrophic factor, is decreased, and this effect is reversed by antidepressant therapy (Roceri et al., 2002). Both BDNF and CREB (cyclic AMP response element binding protein) are proposed to be associated with the anhedonic symptoms and learning-memory impairments observed in stressed animals (Wallace et al., 2009).

The unpredictable chronic mild stress (UCMS) model is an important behavioral model that resembles human depression (Willner, 1997). Chronic stress plays as a predisposing role and is a precipitating factor in humans in certain depression models. In rodents, UCMS has good face validity, as it can elicit depression-like symptoms (Mutlu et al., 2009; Pothion et al., 2004). It is proposed that chronic stress causes behavioral changes such as reduced locomotor activity, reduced food and water intake, decreased responding to reward stimuli (Griffiths et al., 1992) and a degradation in the physical coat state (Ducottet et al., 2003), which are reflective of clinical depression.

In the present study, we investigated the effects of the chronic administration of tianeptine or olanzapine on UCMS-exposed mice compared to mice exposed to a conventional SSRI antidepressant, fluoxetine. To determine the effects of the UCMS regimen and drug therapy, the coat state of the animals was evaluated, splash test, resident intruder test, tail suspension test and novelty suppressed feeding test, which are all frequently used and effective methods for evaluating depression-like behaviors (Binfaré et al., 2010; Mineur et al., 2003; Surget et al., 2008; Yalcın et al., 2005, 2008) were performed. The UCMS-induced changes in stress hormones and proinflammatory cytokines were evaluated by measuring plasma ACTH, TNF-α, and IL-6 levels. The generation of new cells in the adult mammalian brain may significantly modify the pathophysiological processes in neuropsychiatric disorders. To explore the underlying mechanisms of this action, we examined the effects of these drugs on cellular proliferation and differentiation in the adult mouse hippocampus.

Materials and methods

Animals

Male inbred BALB/c ByJ mice (MAM TUBITAK, Gebze, Kocaeli, Turkey) that were 7–8 weeks old at their arrival to the laboratory were used in this study. They were kept in the laboratory for 2 weeks before the onset of the experiments. The animals were assigned to one of two treatment groups: non-stressed mice (controls) and mice subjected to the UCMS procedure. The UCMS treatment continued during the behavioral testing phase; however, care was taken not to apply stressors just before a behavioral test. Non-stressed mice were group-housed (6 mice per cage) during the experiment, while mice of the stressed group were singly housed in cages (length: 268 mm, width: 135 mm, height: 81 mm) from the initiation of the chronic stress until the end of the study. Non-stressed mice were maintained under standard laboratory conditions (12-h light:12-h dark cycle, lights on at 08:00 pm, 21 ± 1 °C) in a separate room. All the animals received food and water ad libitum. For mice, the group-housed condition is preferred to the singly housing condition, as social isolation is highly stressful for mice. Thus, this housing condition should contribute to the chronic stress effects (Arbe et al., 2002; Muscat and Willner, 1992; Spani et al., 2003). All the procedures described in this paper were conducted in accordance with the European Community Council directive for the Ethical Treatment of Animals (86/609/EEC) and with the approval of the Kocaeli University Medical Faculty (10/8-2009) and all the authors.

Experimental groups and drug administration

At the end of the 2-week long drug-free UCMS, the mice were assigned to eight experimental groups (n=8 per group) in a semi-randomized manner such that the initial coat state and body weights were equivalent across all of the groups. Stressed and non-stressed animals were treated with tianeptine (5 mg/kg), olanzapine (2.5 mg/kg), fluoxetine (15 mg/kg/day) or vehicle for 5 weeks. All the drugs were administered intraperitoneally (i.p.) each day at 13 h30 pm in a volume of 0.1 ml/10 g body weight. At the end of the UCMS regimen, the animals were assessed using the splash test, the resident intruder test, the tail suspension test and the novelty suppressed feeding test. UCMS-induced changes in stress hormones and proinflammatory cytokines were examined by measuring plasma ACTH, TNF-α, and IL-6 levels (n=6) in a separate group of animals which were not exposed to behavioral testing. The effects of the examined drugs on cellular proliferation and differentiation in the adult mouse hippocampus were also evaluated (n=5 per group).

Unpredictable chronic mild stress (UCMS) procedure

The UCMS regimen used in this study was based on the procedure originally designed by Willner et al. (1992) and adapted to mice (Ducottet and Belzung, 2004). This stress model consists of repeated, mild physical and psychological stressors. The mice were subjected to different kinds of stressors several times a day for 7 weeks in a chronic, inevitable and unpredictable way. The stressors included damp sawdust, changing the sawdust, placement in an empty cage or an empty cage with water on the bottom (bath), placement in a soiled cage with an aversive odor, social stress (switching the cages), cage tilting (45 °C), predator sounds for 15 min, inversion of the light/dark cycle, lights on for a short time during the dark phase or lights off during the light phase, and confinement in a tube (Table 1). The stressors were administered in a pseudo-random manner and could occur at any time of night or day. The stressor sequence was changed every week to make the stress procedure unpredictable. During the behavioral tests, the stress procedure was slightly modified. The number of stressors applied during the light period was reduced so as not to interfere with the tests. Non-stressed mice were left undisturbed in their home cages. For ethical reasons, the stress procedure did not involve food and water deprivation or immobilization. The mice tested were not subjected to any stressors 12 h before the behavioral tests. In all the experiments, the first 2 drug-free weeks of UCMS were followed by 5 weeks of UCMS during which the mice were treated with drug or vehicle. To determine the effects of the UCMS regimen and drug treatment, we examined the state of the coat in mice and performed the splash, resident intruder, tail suspension and novelty suppressed feeding tests. The open field test was also performed to measure the locomotor activity. For further details on the procedure, see Yalcín et al. (2005). All of the non-stressed animals were isolated 1 day before the open field test to match the condition of the UCMS mice.

Coat state and body weight

Before and during the UCMS, the state of the coat and the body weights of the animals were recorded once a week. The evaluation of the coat state was performed by the assessment of eight different body parts: head (including eyes and nose), neck, dorsal coat, ventral coat, tail, forepaws, hindpaws and genital region (Ducottet et al., 2003; Ducottet and Belzung, 2004). A score of 0 was given for a coat in a good state, and a score of 1 was given for a dirty coat (fur) or piloerection in each of these areas. The sum of these scores gave us an index of the general physical state of a mouse. A dirty state is characterized by a fluffy, greasy, less dense coat or piloerection. The state of the coat was evaluated by observers unaware of the treatment condition of the mice.
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Table 1
The unpredictable chronic mild stress procedure.
Behavioral tests

Splash test

This test was used to evaluate the grooming behavior of the mice. A sucrose solution (10%) was squirted on the dorsal coat of mice in their homecage. The total number of grooming was recorded for 5 min after the vaporization of the sucrose solution (Ducottet and Belzung, 2004). All the mice were then placed in their home cage. The observer was unaware of the treatment conditions.

Resident/intruder test

The resident/intruder test was performed as previously described by Mineur et al. (2003). Non-stressed mice were isolated 48 h before the test, during which time the bedding was not changed to increase the amount of territorial cues within the cages. The mice were tested against a C57/BL6 intruder. The opponent was placed into the cage of the test animal (resident) in such a way that the mice were placed in opposite corners. The cage was then covered with a plastic lid. The test started immediately and lasted for a maximum of 5 min. The number of attacks between the resident and intruder mice was recorded.

Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). The mice were isolated acoustically and visually and suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 5-min period (Binfaré et al., 2009; Mantovani et al., 2003).

Novelty suppressed feeding (NSF) test

The NSF test was a modified version of a previous study (Santarelli et al., 2003). The testing apparatus consisted of a 33 × 33 × 30 cm box. The floor was covered with 2 cm of sawdust. Twelve hours before the test, food was removed from the cages. At the time of testing, a single pellet of food (regular chow) was placed on a white paper positioned in the center of the box, and the animal was placed in the corner. The latency to chew the pellet was recorded within a 5-min period. This test induced a conflicting motivation between the drive to eat the food pellet and the fear of venturing into the arena.

Open field test

The testing apparatus consisted of a 33 × 33 × 30 cm box. The animal was placed in the center of the apparatus, and behaviors were recorded for a period of 5 min using the Ethovision-XT video tracking system. Locomotor activity was evaluated by measuring the total distance traveled in the apparatus and the velocity of the animals. The time spent in the central part of the apparatus was also calculated because in this situation, rodents spontaneously prefer the periphery of the apparatus to the central parts of the open field. Increased time spent in the central part was used as an index of anxiolysis.

Plasma ACTH assay

The animals were sacrificed after 7 weeks of UCMS. Blood samples were collected into 5 cm³ tubes containing EDTA. The samples were centrifuged at 3000 rpm for 10 min. Plasma ACTH levels were analyzed immediately. Other samples were kept at −70 °C in eppendorf tubes for other analyses. Plasma ACTH levels were determined with a chemiluminescent immunometric assay on an IMMULITE 2000 analyzer.

TNF-α and IL-6 determination

Plasma TNF-α and IL-6 levels were measured from the same samples used for plasma hormone testing using enzyme-linked immunosorbent assay (ELISA) kits (Bender Medsystems GmbH, Vienna, Austria) according to the manufacturer’s instructions.

Immunohistochemistry

We examined the ability of chronic treatment with tianeptine, olanzapine, and fluoxetine to increase the number and survival of newly generated cells in the hippocampus of adult male rats. After 5 weeks of drug treatment, the animals were then injected with 5-bromo-2-deoxyuridine (BrdU; Zymed, San Francisco, California, U.S.), a thymidine analogue that is incorporated into DNA as bromouracil during the S phase of the cell cycle (Miller and Nowakowski, 1988), to label newly generated cells. Mice received an intraperitoneal (i.p.) injection of BrdU (75 mg/kg, dissolved in 45% cyclodextrin in saline). Twenty-four hours after, the mice were deeply anesthetized and perfused with 4% paraformaldehyde in phosphate buffer, and the brains were removed and postfixed.

Coronal sections were cut at 5 μm on a microtome. Two sections from each brain approximately 50 μm apart through the anterior hippocampus were processed to reveal BrdU-labeled cells using immunoperoxidase methods. The sections were incubated in 0.2% trypsin (Chemicon International, Inc.) at room temperature and then incubated for 30 min in 2 N HCl. They were labeled using the mouse ABC staining system (sc-56255, 1:250; Santa Cruz Biotechnology, Inc., California). The sections were incubated in block serum for 10 min to reduce nonspecific staining and then incubated in mouse anti-BrdU (sc-56255, 1:250; Santa Cruz Biotechnology, Inc., California) at 4 °C overnight. The sections were incubated with a biotinylated secondary antibody and an avidin and biotinylated enzyme reagent for 30 min at room temperature. The bound antibodies were visualized using 3.3'-diaminobenzidine (DAB) as the chromogen. Finally, the sections were mounted for quantitative analysis. The negative controls consisted of tissue sections incubated without the primary antibody. Images of the stained sections were captured with a Leica DFC290 HD color digital camera mounted on a Leica DM1000 microscope using a 40× objective and stored as TIFF (Tagged Image File Format) images. BrdU-labeled cells in the right and left hippocampus were counted in five randomly chosen square areas (100 × 100 μm).

Drugs

Tianeptine was a gift from Dr. Uzbay (Director of Psychopharmacology Department, Gata University, Ankara, Turkey). Olanzapine was supplied as a gift from Biopharma (Samandira/Kartal/Istanbul, Turkey). Fluoxetine was acquired from Deva (Küçükçekmecе, Istanbul, Turkey). Tianeptine and fluoxetine were dissolved in 0.9% NaCl, while olanzapine was dissolved in 0.9% NaCl with a few drops of 0.1 N HCl. Both drugs were given intraperitoneally (i.p.) at a volume of 0.1 ml per 10 g body weight. The control groups received the same volume of NaCl. Drug doses and dosing times were chosen according to the previous studies (Mutlu et al., 2009, 2011; Ulak et al., 2008).

Statistics

The results from the assessment of the coat state during 6 weeks and body weight at the end of the UCMS regimen, the total grooming time during the splash test, the attack frequency in the resident intruder test, the immobility time in the TST, the latency to reach the food in the NSF test, the locomotion in the open field test, the results of the biochemical and immunohistochemical analysis were compared using two-way ANOVAs with Tukey’s post-hoc test when significant differences were detected. The data are expressed as the mean values ± SEM. Differences were considered to be statistically significant when P was less than 0.05.
Results

Effects of drug treatment on coat state and body weight in the UCMS test

A significant difference between the coat state of the non-stressed group and the UCMS-exposed group was observed. Fig. 1 illustrates the total score from the coat state during 6 weeks of the UCMS regimen. Two-way ANOVA test revealed a significant difference between the groups from the beginning of the first week until the end of the UCMS (F(7,63)=9.02, p<0.0001; F(7,63)=38.26, p<0.0001; F(7,63)=18.53, p<0.0001; F(7,63)=39.66, p<0.0001; F(7,63)=29.28, p<0.0001; F(7,63)=18.48, p<0.0001) (Fig. 1a). We also observed a significant difference between the non-stressed and stressed control groups from the beginning of the second week until the end of the UCMS regimen (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, Fig 1a). All the drugs investigated significantly reversed the UCMS-induced degradation in the coat state. The effects of tianeptine, olanzapine and fluoxetine on the coat state were statistically significant beginning from the fourth week until to the end of the UCMS regimen (p<0.001, Fig 1a).

There was no statistically significant difference in the body weights across all the groups from the beginning of the first week until to the end of the UCMS (F(7,63)=1.27, p>0.05; F(7,63)=1.69, P>0.05; F(7,63)=1.71, P>0.05; F(7,63)=0.60, p>0.05; F(7,63)=1.14, p>0.05; F(7,63)=0.46, p>0.05) (Fig 1b).

Effects of drug treatment on locomotion and anxiety in the open field test

No significant impairment in locomotor activity due to the UCMS regimen or treatment was observed based on the total distance travelled [F(7,63)=2.73, p=0.22, Fig 2a] and the speed of the animals [F(7,63)=1.91, p=0.51, Fig 2b]. There was a significant difference between non-stressed controls and the olanzapine-treated group in center zone duration in the open field test [F(7,63)=7.20, p<0.01, Fig 2c].

Effects of drug treatment on grooming behavior in the splash test

The effects of drug treatment on the total number of grooming in the splash test are shown in (Fig. 3). There was a significant difference between the groups [F(7,63)=7.09, p<0.0001]. Non-stressed vehicle-treated mice groomed significantly more than stressed vehicle-treated mice (p<0.01). Tianeptine, olanzapine and fluoxetine significantly augmented the number of the grooming behavior in stressed mice (p<0.05, p<0.05, and p<0.001, respectively).

Effects of drug treatment on aggression in the resident intruder test

The effects of drug treatment on the attack frequency in the resident intruder test are shown in (Fig. 4). There was a significant difference between the groups [F(7,63)=11.55, p<0.0001]. The attack
Fig. 2. Effects of fluoxetine (15 mg/kg), tianeptine (5 mg/kg) and olanzapine (2.5 mg/kg) (n=8 per group) given intraperitoneally for 35 days on the open field test in mice subjected to unpredictable chronic mild stress (UCMS) and controls: (a) total distance moved; (b) speed; (c) time spent in the center zone. Data are means ± SEM. *p < 0.01, vs stressed control group. nC = non-stressed control (vehicle) group, nF = non-stressed fluoxetine group, nT = non-stressed tianeptine group, nO = non-stressed olanzapine group, sC = stressed control (vehicle) group, sF = stressed fluoxetine group, sT = stressed tianeptine group, sO = stressed olanzapine group.

Fig. 3. Effects of fluoxetine (15 mg/kg), tianeptine (5 mg/kg) and olanzapine (2.5 mg/kg) (n=8 per group) given intraperitoneally for 35 days on total number of grooming in the splash test in mice subjected to unpredictable chronic mild stress (UCMS) and controls. Data are means ± SEM. nC = non-stressed control (vehicle) group, nF = non-stressed fluoxetine group, nT = non-stressed tianeptine group, nO = non-stressed olanzapine group, sC = stressed control (vehicle) group, sF = stressed fluoxetine group, sT = stressed tianeptine group, sO = stressed olanzapine group. *p < 0.01, vs non-stressed vehicle group, #p < 0.05, ##p < 0.001, vs stressed vehicle group.
frequency was significantly increased in the stressed vehicle-treated animals compared to the non-stressed animals (p<0.001). This effect was significantly reversed by fluoxetine, tianeptine and olanzapine treatment (p<0.001 compared to stressed vehicle-treated).

Effects of drug treatment on immobility time in the tail suspension test

The effects of drug treatment on immobility time in the tail suspension test are shown in (Fig. 5). There was a significant difference between the groups [F(7,63)=15.84, p<0.0001]. The immobility time was significantly increased in the stressed vehicle-treated animals compared to the non-stressed animals (p<0.001). The stress-induced increase in immobility time was significantly reversed by fluoxetine, tianeptine and olanzapine (p<0.001, p<0.001, and p<0.01, respectively, compared to stressed vehicle-treated).

Effects of drug treatment on the latency of the first time food is eaten in the NSF test

The effects of drug treatment on the latency until the first time food was eaten in the NSF test are shown in (Fig. 6). There was a significant difference between the groups [F(7,63)=15.84, p<0.0001]. There was no significant difference between the stressed and non-stressed control groups for the latency until the first time food was eaten (p>0.05). Fluoxetine and tianeptine significantly shortened this latency compared to the stressed control group (p<0.01). Olanzapine also shortened this parameter, but it failed to reach to a statistically significant value.

Effects of drug treatment on plasma ACTH, TNF-α and IL-6 levels

As shown in (Table 2), the plasma ACTH levels of UCMS-treated mice were significantly higher than those of the non-stressed control animals [F(7,47)=9.02, p<0.001]. Moreover, a significant increase in plasma TNF-α and IL-6 levels was observed in the mice exposed to UCMS [F(7,47)=5.64 and F(7,47)=9.01, respectively, p<0.001]. The stress-induced increase in plasma ACTH and IL-6 levels were significantly reversed by fluoxetine, tianeptine and olanzapine treatment (p<0.01 or p<0.001, compared to stressed control animals), while the enhanced TNF-α levels were diminished by fluoxetine and tianeptine treatment (p<0.01) but not olanzapine treatment (p>0.05).

Effects of drug treatment on immunohistochemical analysis

Many BrdU-labeled cells were identified in the hippocampi of non-stressed control animals acutely injected with BrdU and then sacrificed 24 h later. In the non-stressed control group, the granule cells were normal with evident nuclei and nucleoli (Fig. 7a). In contrast, the hippocampal region of the stressed mice (sC group) contained degenerating cells (shrunken cells and pyknotic cells) in the dentate gyrus. There were no BrdU-labeled cells in the hippocampi of the stressed animals (Fig. 7b). We observed fewer BrdU-labeled cells in

![Fig. 4. Effects of fluoxetine (15 mg/kg), tianeptine (5 mg/kg) and olanzapine (2.5 mg/kg) (n=8 per group) given intraperitoneally for 35 days on attack frequency in the resident intruder test in mice subjected to unpredictable chronic mild stress (UCMS) and controls. Data are means±SEM. nC = non-stressed control (vehicle) group, nF = non-stressed fluoxetine group, nT = non-stressed tianeptine group, nO = non-stressed olanzapine group, sC = stressed control (vehicle) group, sF = stressed fluoxetine group, sT = stressed tianeptine group, sO = stressed olanzapine group. *p<0.001, vs non-stressed vehicle group, #p<0.001, vs stressed vehicle group.](https://example.com/fig4)

![Fig. 5. Effects of fluoxetine (15 mg/kg), tianeptine (5 mg/kg) and olanzapine (2.5 mg/kg) (n=8 per group) given intraperitoneally for 35 days on immobility time in the tail suspension test in mice subjected to unpredictable chronic mild stress (UCMS) and controls. Data are means±SEM. nC = non-stressed control (vehicle) group, nF = non-stressed fluoxetine group, nT = non-stressed tianeptine group, nO = non-stressed olanzapine group, sC = stressed control (vehicle) group, sF = stressed fluoxetine group, sT = stressed tianeptine group, sO = stressed olanzapine group. *p<0.001, vs non-stressed vehicle group, #p<0.001, vs stressed vehicle group.](https://example.com/fig5)
the hippocampi of olanzapine- and fluoxetine-treated animals. There was a slight, non-significant increase in the total number and density of BrdU-labeled hippocampal cells in the olanzapine- and fluoxetine-treated mice compared to the stressed control group (Figs. 7c,d, 8). Chronic administration of tianeptine significantly increased both the number and the density of BrdU-labeled cells in the hippocampi of the stressed animals (Figs. 7e, 8). There was also a significant difference in the number of newly generated cells between non-stressed controls and the stressed tianeptine-treated group (p<0.05) (Figs. 7e, 8).

**Discussion**

The results of our study reveal that the UCMS regimen produces a significant deterioration of the coat state, and this effect is reversed by the chronic administration of tianeptine, olanzapine and fluoxetine. This procedure had no effect on the body weight or locomotor activity of the animals. All the drugs tested prevented the stress-induced reduction in grooming behavior in the splash test, decreased the attack frequency in the resident intruder test and decreased the immobility time in the tail suspension test. No significant effect was observed between the stressed and non-stressed animals in the novelty suppressed feeding test. Tianeptine, olanzapine and fluoxetine decreased the stress-induced increase in plasma ACTH and IL-6 levels, while the enhanced TNF-α levels were diminished by tianeptine and fluoxetine but not olanzapine treatment. Chronic treatment with tianeptine caused a significant increase in the number of BrdU-incorporating cells in the hippocampus, while the same regimen with olanzapine or fluoxetine had only a small effect on cellular proliferation.

Stress is one of the most validated animal models for generating the depression-like symptoms observed in humans (Papp et al., 1996). Among the animal models used to investigate the effects of antidepressants and the pathophysiology of depression, UCMS is an important behavioral model that reflects human depression in many ways. In studies on rodents, it has been proposed that acute or chronic stress disturbs hippocampal-dependent memory formation (Diamond et al., 2006; Park et al., 2006) and reverses LTP induction (Huang et al., 2005), which is the basis of synaptic plasticity and memory formation (Lynch, 2004). Moreover, chronic stress leads to dendritic deterioration, suppressed neurogenesis, diminished cell proliferation and survival, decreased the neurochemical markers of hippocampal functions, the expression of glutamate transporter and neuronal growth factors (Karten et al., 2005).

It is postulated that antidepressant drugs block or reverse chronic stress-induced cellular changes (Duman, 2004). Tianeptine has been shown to prevent the effects of stress on cognitive functions (McEwen and Olle, 2005) and block the electrophysiological (Vouimba et al., 2005) and morphological/molecular (Fuchs et al., 2005) changes in hippocampal function.

Selective serotonin reuptake inhibitors (Muscat et al., 1992), serotonin and dopamine receptor agonists (Moreau et al., 1996) and atypical antidepressants (Gittos and Papp, 2001) prevented and reversed the behavioral effects of chronic mild stress. Animal studies have revealed that stress induces a coat state degradation, and this effect is reversed by fluoxetine. Moreover, fluoxetine has been shown to prevent the deficit in grooming behavior in the splash test and decrease the attack frequency in the resident-intruder test in stressed animals (Mutlu et al., 2009).

The effect of the drugs used to treat depression and bipolar disorder on learning and memory is related to cAMP response element binding protein (CREB) and the upregulation of neurotrophic factors such as BDNF. These symptoms reveal that the regulation of neuronal plasticity is important for the therapeutic intervention of emotional disorders. CREB and BDNF play important roles in neuronal plasticity (Silva et al., 1998). Stress disrupts BDNF and CREB functions and reverses the effects of drugs used to treat emotional disorders (Roceri et al., 2002; Wallace et al., 2009).

It is postulated that second generation antipsychotics exert better antidepressant effects than typical antipsychotics and are used especially in the augmented therapy of refractory depression (Nemeroff, 2007). Olanzapine is an atypical antipsychotic affecting dopamine

**Table 2**

<table>
<thead>
<tr>
<th>Groups</th>
<th>ACTH (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nC</td>
<td>46.18 ± 8.47</td>
<td>2.46 ± 0.17</td>
<td>171.67 ± 12.33</td>
</tr>
<tr>
<td>nF</td>
<td>57.72 ± 8.23</td>
<td>2.41 ± 0.2</td>
<td>205.41 ± 38.23</td>
</tr>
<tr>
<td>nT</td>
<td>82.28 ± 10.64</td>
<td>2.24 ± 0.09</td>
<td>197.85 ± 37.79</td>
</tr>
<tr>
<td>nO</td>
<td>107.17 ± 26.19</td>
<td>2.66 ± 0.22</td>
<td>205.14 ± 31.37</td>
</tr>
<tr>
<td>sO</td>
<td>213.67 ± 26.07</td>
<td>3.93 ± 0.18</td>
<td>197.75 ± 71.95</td>
</tr>
<tr>
<td>sO</td>
<td>117.33 ± 10.25</td>
<td>2.24 ± 0.11</td>
<td>262.31 ± 63.75</td>
</tr>
<tr>
<td>sF</td>
<td>111.28 ± 10.57</td>
<td>2.48 ± 0.15</td>
<td>392.57 ± 113.24</td>
</tr>
<tr>
<td>sC</td>
<td>109.1 ± 11.48</td>
<td>3.24 ± 0.34</td>
<td>284.91 ± 78.25</td>
</tr>
</tbody>
</table>
(D1, D2, D3 and D4) and serotonin receptors (5HT2A, 5HT2C, 5HT3 and 5HT6). Chronic olanzapine treatment has been shown to exert antidepressant effects in rats exposed to a chronic stress protocol (Marston et al., 2011). It also protected against stress-induced anhedonia in a depression rodent model (Marston et al., 2011). Olanzapine used alone or in combination with fluoxetine significantly improved bipolar depression (Tohen et al., 2003). The mechanism underlying the antidepressant effects of second generation antipsychotics is not fully understood, although there is some clinical evidence for the antidepressant efficacy of these drugs in depression. Compared to other antipsychotics, olanzapine significantly increases plasma BDNF levels. Moreover, olanzapine and clozapine increase BDNF expression in hippocampal neurons (Bai et al., 2003; Luo et al., 2004).

While classic tricyclic antidepressants and SSRIs block serotonin (5-HT) reuptake, tianeptine selectively enhances 5-HT uptake into rat brain synaptosomes (Mennini et al., 1987). The serotonin reuptake enhancer tianeptine has an antidepressant efficacy similar to SSRIs (Wilde and Benfield, 1995). Interestingly, chronic tianeptine administration decreases the increase in plasma ACTH and corticosteroid levels induced by lipopolysaccharides and stress (Delbende et al., 1994). According to these findings, chronic tianeptine therapy could suppress pituitary–adrenal axis responses to stress. Additionally, tianeptine decreases the stress-induced increase in hypothalamic CRF (corticotropin releasing factor) concentrations (Delbende et al., 1994). These reports support the idea that tianeptine has antidepressant effects and regulates the pituitary–adrenal axis and/or CRF neurotransmission in the hypothalamus. Tianeptine has beneficial effects in the amygdala and cortex and could reverse the stress-induced effects on neuronal and synaptic functions. In animal depression models, tianeptine has been shown to reverse chronic stress-induced effects on brain plasticity (Czéh et al., 2001). Tianeptine increases the induction of synaptic plasticity in the hippocampus and amygdala (Vouimba et al., 2003). Chronic tianeptine administration decreases the potentiation of stress-induced aggression and the incidence of stress-induced aggression (Wood et
The antidepressant activity of tianeptine has been reported to be related to central neuronal remodeling and the restoration of neuronal plasticity. In depressed animal models, tianeptine prevents neurodegeneration in response to chronic stress and reverses the decreased hippocampal volume (Fuchs et al., 2002).

Chronic, but not acute, fluoxetine administration leads to the upregulation of BDNF-dependent LTP-associated genes. In previous animal studies, fluoxetine diminished and reversed depressive-like behaviors, exerted emotional changing effects and increased CREB levels in intact rats. The CREB signaling system has been suggested to play a role in the neuronal mechanism of the antidepressant effects of fluoxetine. It has been reported that CREB-expression is upregulated in the prefrontal cortex and hippocampus in response to treatment with antidepressant and antipsychotic drugs (Manji et al., 2001).

The effects of stress on feeding behavior and body weight are still controversial. Although various studies indicate a decrease in these parameters (Haleem and Parveen, 1994), some recent studies indicate an increase or no change in these parameters (Sanchez et al., 1998). In our study, neither the stress regimen nor the investigated drugs had an effect on the body weight of the animals.

Recent studies have demonstrated that stressed animals show a decrease in grooming behavior in the splash test, an increase in aggression in the resident intruder test (Mutlu et al., 2009), and an increase in immobility in the tail suspension test (Binfaré et al., 2010), which is similar to our results. Conflicting results exist for the effects of stress on the NSF test. Some groups report that there is no effect of stress on this test, as found in our study (Surget et al., 2008).

The HPA (hypothalamic–pituitary–adrenocortical) axis is the chief regulator of stress reactions. While several hormones direct stress reactions, often in concert with each other and with some playing more than one role, ACTH is probably one of the most typical stress hormones. During stress, stress hormones are released under control of the HPA axis to help the body cope. Our results reveal that the stress-induced increases in ACTH levels were reversed by drug treatment. The production of IL-6, TNF-α and other proinflammatory cytokines can also be directly stimulated by depression and other negative emotions and stressful experiences (Lutgendorf et al., 1999; Maes et al., 1999). Indeed, both physical and psychological stressors can provoke transient increases in proinflammatory cytokines (DeRijk et al., 1997; Zhou et al., 1993). Additionally, negative emotions contribute to an increased risk for infection, prolonged infection, and delayed wound healing (Glaser et al., 2000; Vedhara et al., 1999), all of which are processes that can fuel sustained proinflammatory cytokine production. Thus, stressors can directly affect the cells of the immune system and modulate the secretion of proinflammatory cytokines. In support of this theory, the secretion of proinflammatory cytokines increased after exposure to chronic stress, and this effect was reversed by drug treatment in our study.

It has been shown that hippocampal CA1 pyramidal cells are selectively vulnerable to stress (Rot et al., 2009). In previous studies, treatment with olanzapine, an atypical antipsychotic drug, caused a marked increase in the number of BrdU-labeled subventricular zone (SVZ) cells (Wakade et al., 2002), while in our study, olanzapine had a partial effect on the density of BrdU-labeled cells in the hippocampus but had no effect on the number of BrdU-labeled cells. Our study shows that tianeptine significantly increases both the number and density of newly generated cells in the CA1 subregion, while fluoxetine and olanzapine have a partial effect. It is possible that new cells induced by fluoxetine, olanzapine and tianeptine treatment might prove to be a beneficial effect of these drugs in behavioral tests.

Conclusion

Fluoxetine, tianeptine and olanzapine reversed the stress-induced effects following the UCMS test and can be used safely in patients with depression. Several mechanisms, including receptors, neurotransmitters, neuronal growth factors and cellular changes, may be responsible from the mechanism of action of antidepressants, and this should be clarified in further studies. The results of this study support the hypothesis that tianeptine can be as effective as fluoxetine for the treatment of depression, in spite of their different mechanisms of action. Moreover, olanzapine could also be used effectively to treat psychotic patients with depression.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References


