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Tianeptine, olanzapine and fluoxetine show similar restoring effects on stress induced molecular changes in mice brain: An FT-IR study

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ABSTRACT

Chronic stress which can cause a variety of disorders and illness ranging from metabolic and cardiovascular to mental leads to alterations in content, structure and dynamics of biomolecules in brain. The determination of stress-induced changes along with the effects of antidepressant treatment on these parameters might bring about more effective therapeutic strategies. In the present study, we investigated unpredictable chronic mild stress (UCMS)-induced changes in biomolecules in mouse brain and the restoring effects of tianeptine (TIA), olanzapine (OLZ) and fluoxetine (FLX) on these variations, by Fourier transform infrared (FT-IR) spectroscopy. The results revealed that chronic stress causes different membrane packing and an increase in lipid peroxidation, membrane fluidity. A significant increment for lipid/protein, C═O/lipid, CH_3/lipid, CH_2/lipid, PO^-_2/lipid, COO^-/lipid and RNA/protein ratios but a significant decrease for lipid/protein ratios were also obtained. Additionally, altered protein secondary structure components were estimated, such as increment in random coils and beta structures. The administration of TIA, OLZ and FLX drugs restored these stress-induced variations except for alterations in protein structure and RNA/protein ratio. This may suggest that these drugs have similar restoring effects on the consequences of stress activity in brain, in spite of the differences in their action mechanisms. All findings might have importance in understanding molecular mechanisms underlying chronic stress and contribute to studies aimed for drug development.

1. Introduction

Stress, a widely used word in our daily life, is generally defined as the responses to severe demands on the body. It has been well established that chronic stress may trigger biochemical mechanisms that can cause a variety of disorders ranging from metabolic and cardiovascular to mental [1], especially when it is followed by anxiety and depression [2]., Chronic stress has been pointed out as one of the main factors for depression although there are also some other risks for its development [3]. According to depression-related stress statistics of World Health Organization (WHO), 121 million people worldwide are estimated to suffer from depression; and by the year of 2030 depression is projected to be the second factor for the cause of disease burden worldwide [4].

Many different types of medications are available for treatment of depression, potentially caused by stress. Among such agents, fluoxetine, also known by trade names Prozac and Sarafem, (FLX), is a selective serotonin (5-HT) reuptake inhibitor (SSRI) which reverses the effects of stress on brain plasticity. This drug is commonly prescribed for treating major depression due to its tolerability and safety [5,6]. A typical antidepressant tianeptine, (brand names Stablon, Coaxil, Tatinol, Tianeurax and Salymbra) (TIA) has an opposite action mechanism to SSRIs. It exerts its action by selectively either enhancing or facilitating 5-HT uptake [7,8]. Both FLX and TIA diminish the neuronal degeneration caused by depression. Olanzapine (originally branded Zyprexa) (OLZ) is an atypical antipsychotic, which is also proposed to have antidepressant-like activity via both serotonergic and dopaminergic receptors [9]. Even though these kinds of agents have been used for many years, 30% of patients do not respond to pharmacological treatment. This may be due to the limited understanding of pathophysiology of stress activity [10]. Thus, further studies are needed to broaden in order to develop novel strategies for stress-related disorders and illness including depression [11].

Stress-based animal models serve for antidepressant drug development. One of these models is unpredictable chronic mild stress (UCMS). Upon exposing to different stressors UCMS-mice/rats exhibit degradation in the physical coat state, a decrease in the grooming behavior and an increase in aggression. All are reflective of clinical depression, most of which can be reversed by antidepressant

Abbreviations: WHO, World Health Organization; FLX, fluoxetine; SSRI, selective serotonin (5-HT) reuptake inhibitor; TIA, tianeptine; OLZ, olanzapine; UCMS, unpredictable chronic mild stress; FT-IR, Fourier transform infrared spectroscopy; KBr, potassium bromide; MRC, mitochondrial respiratory chain; ROS, reactive oxygen species; NO, nitric oxide; AA, arachinoic acid; PC, phosphatidylcholine; PA, phosphatidic acid; SM, spingomyelin; Cer, cerAmide; DAG, diacylglycerol.

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agents, illustrating a strong predictive validity [6,12,13]. Therefore, UCMS model has been accepted as a reliable model to screen antidepressants.

It has been reported that stress can produce some changes in brain structures, which might affect its functions [14–16]. The alterations in content and structure of biomolecules are probably responsible for these observed structural changes in brain. Accordingly, understanding of these variations provides the identification of stress-induced pathologies, which in turn facilitates development of drug therapies to target these defects.

The evaluation and validation of stress target biomarker might imply costly and lengthy processes. An ideal test providing simultaneous information about biomolecules requires poor sample manipulation and it has to be possibly non-invasive, and reliable. Fourier transform infrared (FT-IR) spectroscopy is a suitable method for this purpose, since it has high sensitivity in detecting changes in the functional groups belonging to tissue components such as lipids, proteins, carbohydrates and nucleic acids, simultaneously [17–24]. The shift in peak positions, bandwidth and peak area/intensity parameters gives valuable information, all of which can be used for identification of disease-states in a variety of biological samples such as tissues, membranes, cells and biofluids. Therefore, this technique has been widely employed for diagnosis of several diseases and the effects of selected drugs on disease conditions [17,18,20–23,25–32]. By taking the advantages of FT-IR spectroscopy, we aimed to acquire an overview about how stress affects content and structure of biomolecules in mice brain and the potency of three different drugs (TIA, OLZ and FLX) to restore these variations, which have not been reported previously to our knowledge. In the current study, we obtained relative information about UCMS-induced alterations in the quantity of lipid, protein and RNA molecules, membrane lipid packing, membrane fluidity, membrane order and lipid peroxidation along with the impacts of TIA, OLZ and FLX on these molecular alterations. Moreover, by detailed analysis of Amide I mode we also predicted the changes in protein secondary structures.

2. Material and methods

2.1. Chemicals

TIA, OLZ and FLX were supplied as gift from Gata University, Biopharma and Deva, in Turkey, respectively. Potassium bromide (KBr) was purchased from Sigma Chemical Company (Sigma Chemical Co., St. Louis, MO, USA). All chemicals were used without further purification.

2.2. Animals

Male 7–8 weeks-old BALB/c ByJ mice (MAM TUBITAK, Gebze, Kocaeli, Turkey) weighing 35–45 g were housed five-six per home cages (L30 × W20 × h12.5 cm) in an animal colony facility for 2 weeks before the experiments. At first stage, animals were divided into two groups as unstressed (controls) (nC) and stressed (sC) applied to UCMS procedure. During the experiments unstressed mice were grouped-housed (5 mice per cage) while mice of the stressed group were singly-housed in cages (length: 268 mm, width: 135 mm, height: 81 mm) till the end of the experiments. Control group was maintained in constant room temperature (21 ± 1 °C) under a 12-h light/dark cycle (light onset at 08:00 h) in a separate room. Tap water and food pellets were provided ad libitum. All procedures were in compliance 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.

2.3. Unpredictable chronic mild stress model

The UCMS was applied to all stressed group based on the procedure originally designed by Willner et al. [33] and adapted to mice [34]. 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 (detailed information can be obtained from [6]). 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. Unstressed mice were left undisturbed in their home cages. For ethical reasons, the stress procedure did not involve food and water deprivation or immobilization. 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. 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 Yalçın et al. [35]. All of the unstressed animals were isolated 1 day before the open field test to match the condition of the UCMS mice.

2.4. Experimental groups and drug administration

At the end of the 2-week long drug-free UCMS, the mice were divided into eight experimental groups: unstressed control (nC n = 7), unstressed FLX (nF n = 6), stressed control (sC n = 6) and stressed FLX (sC n = 6), unstressed TIA (nT n = 6), stressed TIA (sT n = 6), unstressed OLZ (nO n = 6) and stressed OLZ (nO n = 5). Stressed and unstressed animals were treated with TIA (5 mg/kg), OLZ (2.5 mg/kg), FLX (15 mg/kg/day) or vehicle for 5 weeks. Before drug administration, TIA and FLX were dissolved in 0.9% NaCl, while OLZ was dissolved in 0.9% NaCl with a few drops of 0.1 N HCl. All 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 [6,36]. At the end of the drug administration, all brain tissues were decipated and kept at −80 °C until FT-IR study.

2.5. Sample preparation for FT-IR study

For FT-IR spectroscopic study earlier works studied dried samples were followed [19,24,37–39]. Briefly, brain tissues were dried in using a lyophilizer for 24 h to remove free and unbound water. Dried samples were first ground into fine particles using mortar and pestle from agate (4 cm diameter bowl), which is non-absorbing in the infrared spectroscopic region [40]. 1 mg of each sample was then mixed with 100 mg KBr and ground for 2–3 min until crystallites could no longer be seen. This procedure may cause KBr to absorb water from atmosphere due to its hygroscopic feature. Since water content within the sample interferes with IR spectra, the sample containing KBr must be freshly dried. For this reason, the mixtures were re-dried in lyophilizer for an additional 2 h. Then, KBr based pellets were prepared by establishing pressure of 100 kg/cm^2 (1200 psi) for 8 min.

2.6. FT-IR spectroscopic study and data analysis

A Perkin Elmer Spectrum One spectrometer (Perkin Elmer Inc., Norwalk, CT, USA) equipped with a DTGS detector was used to acquire IR spectra. The sample compartment in the FT-IR spectrometer was