Experimental and Toxicologic Pathology 69 (2017) 564–574

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.com/locate/etp

Protective effects of melatonin on long-term administration of fluoxetine in rats

Majid Khaksar[a,c], Ahmad Oryan[a], Mansour Sayyari[a], Aysa Rezabakhsh[b,c,1,*], Reza Rahbarghazi[c,d,1,**]

[a] Department of Pathobiology, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Keywords:
Fluoxetine
Melatonin
Pathology
Biochemical–hematological parameters

ABSTRACT

The degree and consequence of tissue injury are highly regarded during long-term exposure to selective antidepressant fluoxetine. Melatonin has been shown to palliate different lesions by scavenging free radicals, but its role in the reduction of the fluoxetine-induced injuries has been little known.

Thirty-six mature male Wistar rats were randomly assigned into control and experimental groups. The experimental rats were included as following; 24 mg/kg/bw fluoxetine for 4 weeks; 1 mg/kg/bw melatonin for 4 weeks; fluoxetine + 1-week melatonin, fluoxetine + 2-week melatonin and fluoxetine + 4-week melatonin. In the current experiment, we investigated weight gain, hematological and biochemical parameters, pathological injuries and oxidative status.

We noted the positive effect of melatonin in weight loss of fluoxetine-treated rats ($p < 0.05$). The significant reduction of superoxide dismutase, glutathione peroxidase, catalase activities in blood, liver, and kidneys and changes in serum total antioxidant capacity caused by fluoxetine were reversed by melatonin ($p < 0.05$). Melatonin reduced the increased lipid peroxidation and transaminase activity in rats received fluoxetine ($p < 0.05$). We also showed the potency of fluoxetine in inducing leukopenia, thrombocytopenia and hypochromic and macrocytic anemia which was blunted by melatonin. Both RBCs and platelets indices were also corrected. Rats received melatonin in combination with fluoxetine showed a reduction in the severity of degeneration and inflammatory changes in different tissues, brain, heart, liver, lungs, testes and kidneys as compared to the fluoxetine group.

Therefore, melatonin fundamentally reversed the side effects of fluoxetine in the rat model which is comparable to human medicine.

1. Introduction

Due to the widespread occurrence of depression, not limited exclusively to mental suffering individuals, different psychopharmacological and antidepressant agents have been prescribed extensively in human medicine (McHenry, 2006). In 1988, the first selective serotonin reuptake inhibitors (SSRIs), named fluoxetine (Prozac), were introduced to human medicine. This drug has potential to increase 5-brain HT level in the serotonergic synaptic space (Anderson, 1998; Fuller, 1995). In comparison to any other available antidepressant, fluoxetine possesses superior side effects because of its selectivity for serotonin receptors, especially following repeated administrations (Ferguson, 2001). Noticeably, the reductions of both appetite and body weight are considerable among its therapeutic effects (Chojnacki et al., 2015). As a consequence, prolonged use of fluoxetine contributes to desensitization of presynaptic 5-HT_{1A} and 5-HT_{1B} auto-receptors with augmentation of extracellular 5-HT (Blier and De Montigny, 1994). Many longitudinal and retrospective investigations revealed the occurrence of tissue toxicity such as sexual dysfunction, hepatotoxicity, nephrotoxicity and *etc.* following fluoxetine therapy (Inkielewicz-Stępniak, 2011;

* Corresponding author at:Faculty of Pharmacy, Tabriz University of Medical Sciences, Daneshgah St., Tabriz 51664-14766, Iran.
** Corresponding author at:Tabriz University of Medical Sciences, Imam Reza St., Daneshgah St., Tabriz 51666-14756, Iran. Tel.: +984133373879; fax: +984133363870.
E-mail addresses: majid.khaksar1365@yahoo.com, m.khaksar@shirazu.ac.ir (M. Khaksar), Oryan@shirazu.ac.ir (A. Oryan), Sayari32@yahoo.com (M. Sayyari), aysapharma.rezabakhsh@gmail.com (A. Rezabakhsh), Rezarahbardvm@gmail.com (R. Rahbarghazi).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.etp.2017.05.002
Received 6 October 2016; Received in revised form 5 May 2017; Accepted 5 May 2017

Modabbernia et al., 2012). The administration of this drug for pregnant women has been shown to be associated with aberrant or insufficient intrauterine growth and impaired somatosensory and psychomotor maturation in the fetus (Berg et al., 2013). Up to now, different underlying mechanisms related to the fluoxetine toxicity have been described (Herbet et al., 2014; Inkielewicz-Stępniak, 2011). For example, an excessive production of free radicals along with the inability of the antioxidant response system and accelerated lipid and protein peroxidation have been reported in rats receiving fluoxetine, orally (Inkielewicz-Stępniak, 2011). In a work done by Herbet and co-workers, they acclaimed that a 14-day treatment with 10 mg/kg fluoxetine increased the parameters of oxidative stress in rats (Herbet et al., 2014). Unlike some harmful effects, Corbett et al. however, showed a 10-fold increase in the rate of brain neurogenesis in the rat model when the animals were treated with fluoxetine (Corbett et al., 2015). A few studies exist regarding the effects of fluoxetine on tissues other than the brain. With this background, it seems that further investigations are essential to elucidate different effects of serotonin reuptake inhibitors in smart *in vitro* and *in vivo* models.

Melatonin is a multifunctional indolamine that is preferentially produced in the pineal gland, although many researchers discovered the broad-spectrum effective role of melatonin biosynthesis in other organs such as skin, retina, and gut (Gonzalez-Arto et al., 2016; J Reiter et al., 2013; Maldonado et al., 2010). It has been characterized that melatonin acts synergistically with serotonin in different physiological phenomena. For instance, melatonin could regulate carbohydrate and lipid metabolism, appetite, and possesses remarkable anti-depressant activity (Wolden-Hanson et al., 2000; Zanuto et al., 2013). Administration of melatonin with antidepressants has been suggested for treatment of depressive disorders, especially in postmenopausal women (Hall and Steiner, 2013; Targum et al., 2015; Toffol et al., 2014). Among the numerous benefits promised, melatonin is publicly known as the best anti-oxidants, since it responds easily to free radicals and has the characteristic of free radical scavenging activity (Manchester et al., 2015; Zhang and Zhang, 2014). Unlike to other antioxidants, melatonin does not enter redox cycling without any potent pro-oxidants capacity (Bizzarri et al., 2013). All the above-mentioned realities pinpoint the necessity of melatonin administration in the treatment of complex depression disorders.

In the current study, we have explored the possibility of using melatonin in reducing fluoxetine side effects in the model of rat. Therefore, we aimed to evaluate the effects of the combined administration of fluoxetine and melatonin on biochemical and hematological parameters and oxidative status. Amelioration of the pathological changes induced by fluoxetine was monitored in different tissues. The results of the current experiment could shed lights to neutralize the exacerbating effect of fluoxetine by a natural hormone.

2. Material and methods

2.1. Animals and ethical issues

Thirty-six 1-month male Wistar rats at the normal weight, 85 ± 10 g, were purchased from Razi Institute (Karaj, Iran). The rats were allowed to acclimate to the convenient housing condition with standard temperature (20–24 °C), 12 h light/dark cycle on a 12-h light period between 6:00 a.m. and 6:00 p.m. and free access to feed. The daily time point administration refers to melatonin and fluoxetine at 6:00 a.m. before the light was switched on. After finishing treatment, rats in each group were euthanized immediately with high doses of Ketamine and Xylazine combination. All implementations of the current experiment were conducted in accordance with the previously published NIH standards (Publication No. 85-23, revised 1996) and approved by the Animal Care Committee of Tabriz University of Medical Sciences.

2.2. Experimental procedure

Six different protocols were used to perform the *in vivo* assay. Protocol I: control rats (given 1 ml of normal saline orally for a period of 4 weeks); Protocol II: the rats only received fluoxetine (24 mg/kg/bw; Cat no: F132, Sigma) (Inkielewicz-Stępniak, 2011) for over a course of 4 weeks; Protocol III: the rats received 24 mg/kg/bw fluoxetine for 4 weeks and 1 mg/kg/bw melatonin (Kireev et al., 2008) (Cat. no.: M5250, Sigma) solution during the first week; Protocol IV: the rats received 24 mg/kg/bw fluoxetine for 4 weeks with 2-weeks administration of 1 mg/kg/bw melatonin solution; Protocol V: the rats received 24 mg/kg/bw fluoxetine for 4 weeks with 4-weeks administration of 1 mg/kg/bw melatonin solution and finally Protocol VI: the rat received only 1 mg/kg/bw melatonin for 4 weeks. Drugs of each group were mixed by sterile normal saline and administrated once daily by using a gastric tube. Each group was included six rats.

2.3. Weight change assay

To explore the side effects of fluoxetine on weight gain/loss, the rats were weighed at the beginning and end to the experiment. Thereafter, differences in the weight change were calculated.

2.4. The procedure of sample preparation

To analyze the possible effects of fluoxetine and melatonin administration in *in vivo* model of rats, blood, serum and plasma samples, as well as different tissues lysates, were provided in accordance with manufacturers' instructions. For hematological examinations, blood samples were taken from the jugular vein and pooled in tubes containing EDTA. Total blood cell counts with the percent of cell component were determined by using an automated hematology analyzer (Sysmex KX-21N). To harvest serum from each rat, the blood samples were allowed to clot in sterile tubes and centrifuged at 3000 rpm for 15 min. The supernatant sera from each sample were then collected and stored at −20 °C until use. Different chromogenic assay for each sample was performed using Alcyon 300 auto-analyzer (Abbott Co.). To harvest the tissue samples, 2 g of either hepatic or renal tissues were weighed, homogenized in the presence of a solution of 1.5% KOH and then centrifuged at 3000 rpm for 10 min. The supernatant fluids were subjected to both hematological and biochemical analysis.

2.5. Measurement of the enzymatic oxidative status response in plasma, renal and hepatic niches

To assess the enzymatic antioxidant response, the activities of enzymes within the antioxidant systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat) were monitored either in blood, kidneys, and liver. Shortly, the total activity of these enzymes was estimated in the plasma samples and tissue lysates, using Randox kit according to the manufacturer's instruction (Cat. no.: SD125; RS504; Antrim Co.).

2.6. Measurement of lipid peroxidation by TBARS assay

The Lipid peroxidation end-product status was assessed based on the serum and tissue malondialdehyde (MDA) level and subsequent generation of MDA-2-thiobarbituric acid (TBA) reactive substances as previously described (Malekinejad et al., 2012). A panel serial dilution of the standard solution of MDA including 0.5, 1, 2, 4, 8 and 12 nmol/ml of 1,1,3,3-tetraethoxypropane was prepared. Then, 500 µl of the serum from each group was mixed with 3 ml of 1% phosphoric acid, vortexed for 5 min and 1 ml of 0.67% thiobarbituric acid overlaid and incubated in boiling water for 45 min. After cooling, 3 ml of *n*-butanol solution was added into the samples, mixed and centrifuged at 3000 rpm for 10 min. Ultimately, the absorbance of MDA − TBA adduct