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The effect of olanzapine on IGF-1/IGF-1R and volumes in the prefrontal cortex and hippocampus in a model of bipolar mania

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Summary

Background: *The aim of the study is to determine the changes in insulin-like growth factor 1 (IGF-1) and its receptor (IGF-1R) in the emergence of bipolar disorder and its treatment with olanzapine.*

Subjects and Methods: *Of all 48 adult male albino wistar rats, the control group (n=12) saline, ketamine group (n=12) ketamine (25mg/kg), olanzapine group (n=12) olanzapine (2mg/kg), ketamine+olanzapine group (n=12) ketamine (25 mg/kg) was administered once a day/14 days. Olanzapine (2mg/kg) was administered once a day to the ketamine+olanzapine group between 8 and 14 days. Volume, IGF-1, and IGF-1R gene expression and protein levels were measured in the prefrontal cortex and hippocampus.*

Results: *In the prefrontal cortex, there was a decrease in volume, IGF-1R gene expression, and protein levels in the ketamine group, an increase in IGF-1 gene expression and protein levels in the olanzapine group; an increase in IGF-1R gene expression, IGF-1, and IGF-1R protein levels in the ketamine+olanzapine group. In hippocampus, there was a decrease in volume, IGF-1 gene expression and protein levels in ketamine group, a decrease in volume and IGF-1 protein levels, increase in IGF-1R gene expression and protein levels in olanzapine group; a decrease in volume, IGF-1 protein levels and IGF-1R gene expression and protein levels in ketamine+olanzapine group.*

Conclusions: *One of the underlying causes of bipolar disorder may be changes in the IGF-1 and IGF-1R regions in the prefrontal cortex and hippocampus. Olanzapine has a neuroprotective effect by increasing IGF-1 and IGF-1R levels in the prefrontal cortex, and a neurodegenerative effect by decreasing IGF-1 and IGF-1R levels in the hippocampus.*

Keywords: *Olanzapine, IGF-1, IGF-1R, Prefrontal cortex, Hippocampus*

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INTRODUCTION

Bipolar disorder (BD) is a mood disorder characterized by recurrent episodes of mania, hypomania, depression, or mixed episodes, a chronic course, leading to significant impairment in occupational, familial, and social functionality (Maj et al., 2002). With a global prevalence of up to 4.8%, BD is a disease that reduces the quality of life more than major neurological diseases or cancer (Merikangas et al., 2011). Although it has been shown in previous studies that BD is associated with increased lateral ventricle volume (Arnone et al., 2009; Hibar et al., 2016) and globus pallidus volume (Arnone et al., 2009), decreased brain and prefrontal lobe (Arnone et al. 2009), hippocampus (HC) (Hibar et al., 2016, 2018; Otten & Meeter, 2015), amygdala and thalamus volumes (Hibar et al., 2016), it is not fully understood which changes in which parts of the brain are effective in the onset of bipolar disorder. Olanzapine, a secondary antipsychotic drug used in the treatment of the disease, is a drug that has also found use in other psychiatric diseases after it was approved by the Food and Drug Administration in the treatment of schizophrenia and BD mania and was released to the market (Karamustafalıoğlu, 2018). Olanzapine primarily targets dopaminergic and serotonergic pathways. It acts as an antagonist at dopamine D2 receptors in the mesolimbic pathway, inhibiting post-synaptic receptor activation by dopamine. Due to its low-affinity binding, olanzapine readily dissociates from the receptor,

Abbreviations

B2M: Beta-2-Microglobulin
BD: Bipolar disorder
HC: Hippocampus
IGF-1: Insulin-like growth factor-1
IGF-1R: Insulin-like growth factor-1 receptor
Ket: Ketamine
Olz: Olanzapine
PFC: Prefrontal cortex
qPCR: Quantitative Polymerase Chain Reaction

permitting normal dopaminergic transmission. By antagonizing D2 receptors, olanzapine alleviates positive symptoms such as hallucinations, delusions, and disorganized thought and behavior. Similarly, it antagonizes serotonin 5HT2A receptors in the frontal cortex. This serotonergic modulation ameliorates negative symptoms, including anhedonia, blunted affect, alogia, avolition, and attentional deficits (Tollens et al., 2018). Although It is thought that its antipsychotic effect is mediated by antagonism of serotonin and dopamine receptors, the exact mechanism of action is not known (Bhana & Perry, 2001).

The neurobiological effects of olanzapine remain incompletely understood. While some evidence suggests neurodegenerative consequences, such as neurotoxicity in hypothalamic neurons (Boz et al., 2020), other studies indicate that olanzapine may possess neuroprotective properties (Koprivica et al., 2011; Yang & Lung, 2011). What underlying mechanism could account for this potential neuroprotective effect? Given their established role in neuronal development and survival, one plausible hypothesis involves Insulin-like Growth Factor-1 (IGF-1) and its receptor. Insulin-like growth factor-1 (IGF-1) is a member of the IGF family. The IGF-1 mature protein is a 70 amino acid peptide with structural similarity to insulin. The IGF-1 peptide is encoded by a single IGF-1 gene of six exons. It is mainly expressed in the central nervous system, especially in the central nervous system cell types (Bach et al., 1991; Bondy & Lee, 1993) found in the cortex, HC, cerebellum, and hypothalamus, and peripheral tissues such as the liver (Le Roith, 2003). IGF-1 binds to high-affinity IGF-1 binding proteins both in circulation and in tissues, and this binding modulates the interactions between IGF-1 and its receptor, prolonging the half-life of IGF-1. The biological effects of IGF-1 are mediated by the insulin-like growth factor-1 receptor (IGF-1R), a transmembrane tyrosine kinase composed of two alpha and two beta chains. IGF-1R, a heterotetrameric glycoprotein, is expressed in both neural stem cells and all nerve cells throughout life (Baron-Van Evercooren et al., 1991; Popken et al., 2005). IGF-1 binding to IGF-1R in neurons and astrocytes leads to IGF-1R activation, resulting in tyrosine kinase activity in β-subunits, autophosphorylation, and activation of downstream signals (LeRoith et al., 1995).

IGF-1 has an important role in neurogenesis. In neural stem cells, IGF-1 is associated with increased cell proliferation and extended lifespan (Supeno et al., 2013). It also generates an important anti-apoptotic signal in differentiated neurons (Chrysis et al., 2001; Hodge et al., 2007). IGF-1 has profound effects on the expansion of neuron progenitors (Popken et al., 2004), differentiation and maturation of neurons (Aberg et al., 2000; Brooker et al., 2000; O'Kusky et al., 2000; Trejo et al., 2001; Vicario-Abejón et al., 2003; Otaegi et al., 2006; Carlson et al., 2014; Zhang et al., 2014a; Yuan et al., 2015), development of astrocytes and oligodendrocytes (Ye & D'Ercole, 2006; O'Kusky & Ye, 2012), differentiation of neural progenitors into myelin-producing mature oligodendrocytes, stimulates proliferation and differentiation of astrocytes under physiological conditions (Cao et al., 2003; Ye et al., 2004). In addition, it has been reported that increased IGF-1 expression increases brain size (Popken et al., 2004; Ye et al., 2002).

Studies investigating the effect of olanzapine on IGF-1 are insufficient both in number and in the regions examined in the brain. In a study with endothelial cell/adipose-derived stromal cell co-culture, olanzapine administration increased IGF-1 mRNA expression (Xue et al., 2017). Similarly, it was reported that olanzapine ameliorated the neuropathological changes in C57BL/6 mice exposed to cuprizone and increased the expression of IGF-1 in the frontal cortex (Zhang et al., 2014b).

Based on this information, this study aims to examine the relationship between IGF-1 and IGF-1R levels in the prefrontal cortex (PFC) and HC in the occurrence of bipolar disorder, and also to examine the effect of olanzapine on IGF-1 and IGF-1R.

SUBJECTS AND METHODS

Animal experimentation

This study was conducted with the permission of Ondokuz Mayıs University Animal Ethics Committee (Project Acceptance Number: 2020-23). For this purpose, a total of 48 adult male Wistar albino rats, aged 2–3 months and weighing 200–300 g, were utilized.The rats were housed in standard cages with wood shavings as bedding, and ad libitum was provided access to standard rat pellets (Bil-Yem Co., Ankara, Turkey) and water. Lighting in the experimental environment was programmed as 12 h day and 12 h night, and the temperature was fixed at 22 ± 1 °C. The rats were weighed before and after the experiment. Of all 48 adult male albino wistar rats, the control group (n=12) saline (0.5 ml), ketamine group (n=12) ketamine (Keta-Control, Serial No: 200697) (0.5 ml,25 mg/kg), olanzapine group (n=12) olanzapine (Acros Organics, CAS Number:132539-06-1) (0.5 ml,2 mg/kg), ketamine+olanzapine group (n=12) ketamine (0.5 ml, 25 mg/kg) was administered intraperitoneally once a day for 14 days. In addition to ketamine administration, olanzapine (0.5 ml,2 mg/kg) was administered daily to

the ketamine+olanzapine group between 8 and 14 days. Ketamine doses used to create a bipolar mania model in rats were reported by Canever et al. (2010) and have been selected taking into account their work. Olanzapine doses were determined by considering the study of Bardgett et al., (2002). To detect occurrence of mania on the 15th day, a single dose of ketamine (0.5 ml, 25 mg/kg) was injected into the rats in ketamine group and a single dose of 0.5 ml saline was injected into the rats in the control group and 30 minute later, locomotor activity was assessed using the "open field test". Then, each rat was anesthetized with 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine intraperitoneally, and all rats were decapitated after reperfusion with saline. PFC and HC were removed to measure volume, gene expression, and protein levels. The PFC and HC of the right hemisphere were taken for gene expression analysis and protein levels measurement and stored at -80 °C until the study day. For the histopathological examination, after the perfusion procedure, the left hemisphere was dissected and placed in 10% formalin for post-fixation for 10 days.

Quantitative Polymerase Chain Reaction (qPCR)

PFC and HC samples were homogenized with liquid nitrogen. Using FavorPrepTM Tissue Total RNA Mini Kit (Cat. No: FATRK 001-1) and Trizol (ABP Biosciences, Cat. No: FP312), RNA isolation was performed. The quality and quantity of RNAs were evaluated by the Nanodrop spectrophotometer. Using the iScript cDNA Synthesis Kit (BIORAD, Cat. No: 1708891), mRNAs were converted to cDNA. The IGF-1 and IGF-1R gene expression were measured via qPCR. The primer sequences of target genes are given in Table 1.

Tissue processing and sectioning

After the fixation period, the tissues were embedded in paraffin, and 20 μm sections were obtained using a microtome (Leica RM2245, Nussloch, Germany) in a systematic random sampling manner. Afterward, the deparaffinized sections were stained with 0.1% cresyl violet (C5042-10 gr, Sigma-Aldrich, Germany) for histopathological evaluation and stereological analysis.

Stereological analysis

After fixation of brain tissues and routine histological tissue processing, 20 μm-thick sections were taken from the paraffin blocks obtained with a 1/10 sampling rate according to the systematic random sampling rule of stereology in the coronal plane using a microtome. An average of 45 sections was obtained per subject. HC and PFC volumes were calculated using a point counting grid in light microscopic pictures at x10 magnification obtained from sections of the brain hemisphere stained with cresyl violet. First, the PFC and HC regions' boundaries were determined with the help of an atlas (Paxinos & Watson, 2007). The coordinates for the HC region were Bregma -1.92 mm to -6.48 mm and interaural 7.08 mm to 2.52 mm. The coordinates for the analyzed PFC region were Bregma 5.16 mm to 2.52 mm and interaural 14.16 mm to 11.52 mm. The hippocampal parts, including CA1, CA2, CA3, and DG regions, were the basis for HC volume estimations. The basis of the point counting grid used in the stereological analysis is based on the Cavalieri principle. Stereology is an unbiased morphoquantitative method that statistically evaluates morphological changes in tissue based on data obtained from two-dimensional images of tissues (Peterson & Jones, 1993; Deniz et al., 2018). The Cavalieri principle is a reliable and unbiased method of measuring the total volume of any building and the volume of the building components separately, and obtaining the proportional value of the building components to each other or the whole structure. In this method, it is essential that each section faces the same surface and is parallel (Gundersen & Jensen, 1987).

The pilot study determined the frequency of dots in the dotted area scale used in volume measurement and the cross-section sampling rate, considering the appropriate error coefficient (CE≤0.05). The coefficients of variation (CV≤0.2) for HC volume and PFC volume were acceptable.

Table 1 The primer sequences of target genes

Gene	Forward primer	Reverse primer
B2M	AGCAGGTTCCTCAAACAAGG	TTCTGCCTTGGAGTCCTTTC
IGF-1	AACCTGCAAAACATCGGAAC	GCAGCCAAAATTCAGAGAGG
IGF-1R	GACAGTGAATGAGGCTGCAA	CCAGCCATCTGGATCATCTT

Immunohistochemical analysis

IGF-1 and IGF-1R proteins in the brain were evaluated immunohistochemically in the cornu ammonis region of the HC and PFC. Thin paraffin sections of 7 μm thickness obtained from each rat brain were deparaffinized, followed by IGF-1 (Cat No: E-AB-40014, Elabscience, USA) (1:100) and IGF-1R (Cat No: bs-0227R, Bioss, USA) (1:200) immunohistochemical staining was performed using antibodies. The sections were treated with 3% H_2O_2 after deparaffinization during the immunohistochemical analysis. Sections washed with phosphate buffer and then boiled in citrate buffer in an 800-Watt microwave oven (1:10, Thermo Fisher Scientific, UK) to reveal epitopes. Sections were incubated with protein block to avoid non-specific staining. Sections washed with phosphate buffer were incubated overnight in the primary antibody at +4 ˚C. Then, the reaction consisting of sections incubated at room temperature with biotinylated antibody and streptavidin peroxidase enzyme, respectively, was demonstrated with AEC chromogen. Intermediate washes were done with phosphate buffer. Mayer hematoxylin (Abcam, USA) was used for reverse staining. Brown-stained nuclei were considered a positive reaction.

Statistical analysis

Relative expression software (REST) QIAGEN 2009 analysis used for relative gene expression. Beta-2-Microglobulin (B2M) was accepted as a reference gene, and the control groups were accepted as calibrators. $p<0.05$ was accepted as significant.

The data obtained from the open field test, stereological (volume measurement), and immunohistochemical examination (IGF-1 and IGF-1R protein levels) in the prefrontal cortex and hippocampus were statistically evaluated using SPSS 22.0 (Statistical Package for Social Sciences) software. The normal distribution of the data was tested using the Shapiro-Wilk normality test. While open field test data did not show normal distribution, IGF-1 and IGF-1R levels showed normal distribution. Kruskal-Wallis Test and Mann-Whitney U Test statistical analyses were used for data that did not conform to normal distribution. One-way ANOVA and Post-Hoc Tukey and Tamhane tests were used for data compatible with normal distribution. $p<0.05$ was considered statistically significant.

RESULTS

Open Field Test

In the open field test, a significant increase was found in the ketamine group compared to the control group in the number of square crossings (p=0.021), the number of crossings from the center (p=0.031), and the number of rearing in rats (p=0.000) (Table 2).

qPCR

In PFC, while IGF-1R gene expression levels decreased by 0.277-fold in the ketamine group (p=0.001), IGF-1 gene expression levels increased by 2.082-fold in the olanzapine group (p=0.002), and IGF-1R gene expression levels increased by 1.431-fold in the ketamine+olanzapine group (p=0.037) were detected (Fig. 1).

In HC, IGF-1 gene expression levels decreased by 0.592-fold in the ketamine group (p=0.002), IGF-1R gene expression levels increased by 3.712-fold in the olanzapine group (p=0.000), and IGF-1R gene expression levels decreased by 0.003-fold in the ketamine+olanzapine group (p=0.000) were detected (Fig. 2).

Histological stereology

A decrease (p=0.000) was detected in the PFC cortex volume in the ketamine group only, and a decrease in HC volume was detected in all three groups (control vs ketamine p=0.000, control vs olz p=0.000, control vs ket+olz p=0.000) (Table 3, Fig. 3, Fig. 4).

Table 2 Open Field Test

Number	Groups		p
	Control	Ketamine	
Square Crossing	22,25+24,95	36,75+12,76	0,021
Crossing from the center	0,16+0,38	0,75+0,75	0,031
Rearing up	0,33+0,49	2,58+1,24	0,000
Defecation	2,08+2,15	2,33+1,55	0,423

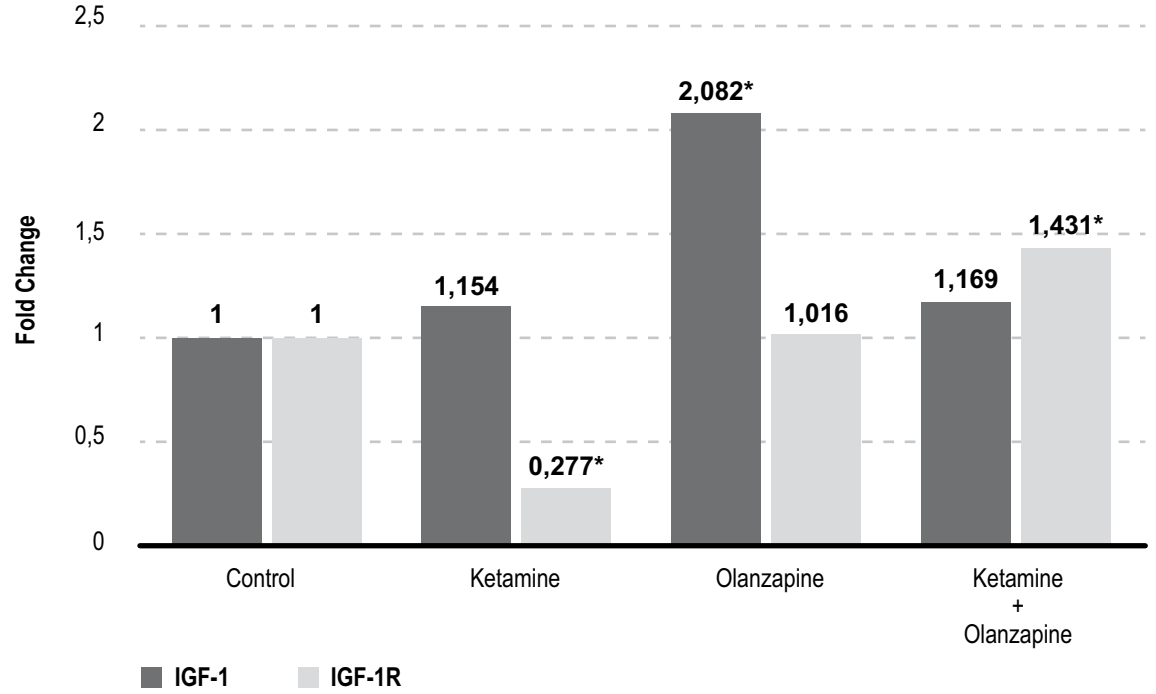

Figure 1 Gene expression changes of IGF-1 and IGF-1R in PFC. * p< 0.05

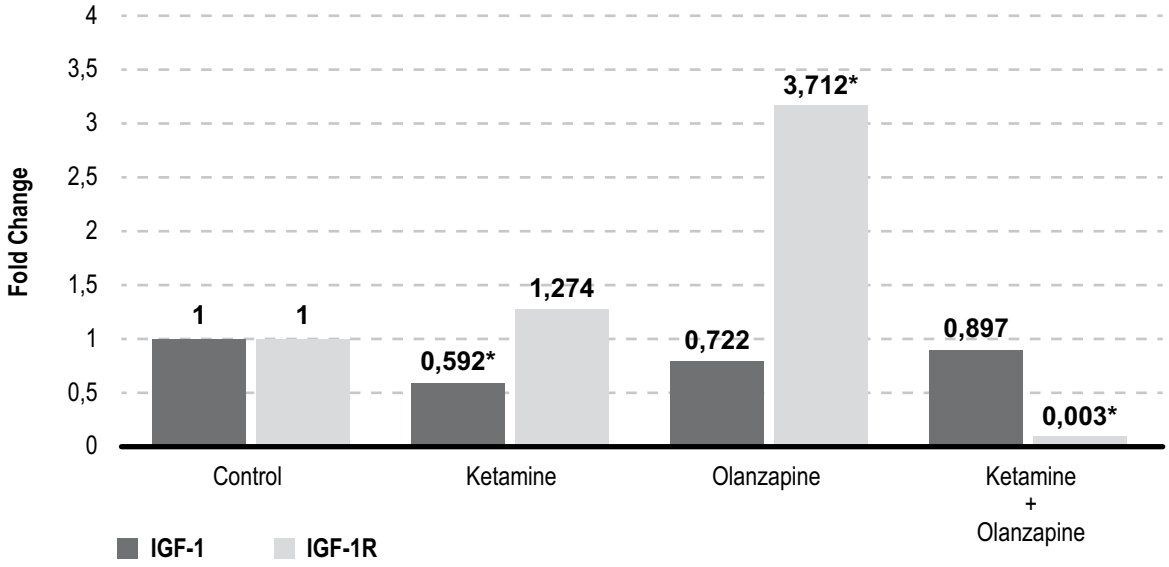

Figure 2 Gene expression changes of IGF-1 and IGF-1R in HC. * p< 0.05

Table 3 Histological stereology results of PFC and HC

Tissue volume (mm^3)	Control	Ketamine	Olz	Ket+Olz	p
PFC	66.55±0.43	62.08±1.31[a]	67.05±0.74	67.17±0.66	0.000
HC	9.85±0.74[b]	8.14±0.05	8.08±0.12	8.54±0.21	0.000

a post-hoc Tukey control vs ketamine p=0.000
b post-hoc Tukey control vs ketamine p=0.000, control vs olz p=0.000, kontrol vs ket+olz p=0.000

Immunohistochemical protein level analysis

In PFC, while IGF-1R protein levels decreased in the ketamine group (p=0.000), increased IGF-1 protein levels in the olanzapine group (p=0.000) were detected. Also, increased IGF-1 (p=0.031) and IGF-1R (p=0.000) protein levels in the ketamine+olanzapine group were detected (Table 4, Fig. 5, Fig. 7, Fig. 8).

There was a decrease in IGF-1 protein levels in the HC in all three groups (control vs ketamine p=0.000, control vs olz p=0.011, control vs ket+olz p=0.035). IGF-1R protein levels in this region were increased (p=0.000) in the olanzapine group and decreased (p=0.000) in the ketamine+olanzapine group (Table 4, Fig. 6, Fig. 9, Fig. 10).

DISCUSSION

In this study, we evaluated the volume, IGF-1 gene expression and protein levels, IGF-1R gene expression and protein levels in the PFC and HC in rat groups in which a bipolar mania model was induced with ketamine. The experimental groups (ketamine, olanzapine, and ketamine + olanzapine) were compared with the control group.

While a significant decrease was observed in the PFC volume in the ketamine group compared to the control, there was no significant change in IGF-1 gene expression and protein levels. However, a significant decrease was detected in IGF-1R gene expression and protein levels. In

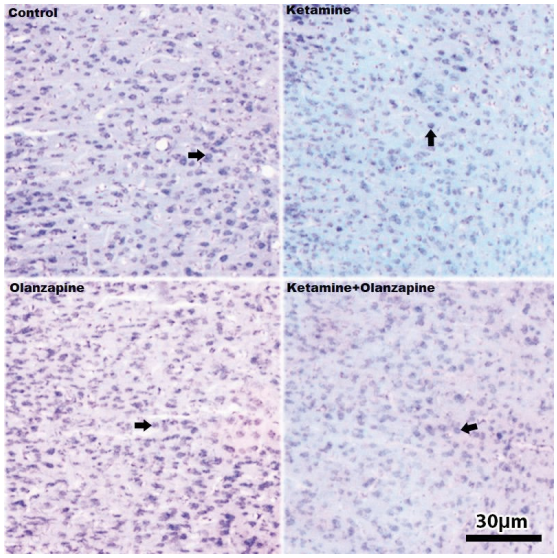

Figure 3 Representative general view of the PFC regions with cresyl violet staining of the study groups at high magnification. Arrows indicate neurons located in respective regions

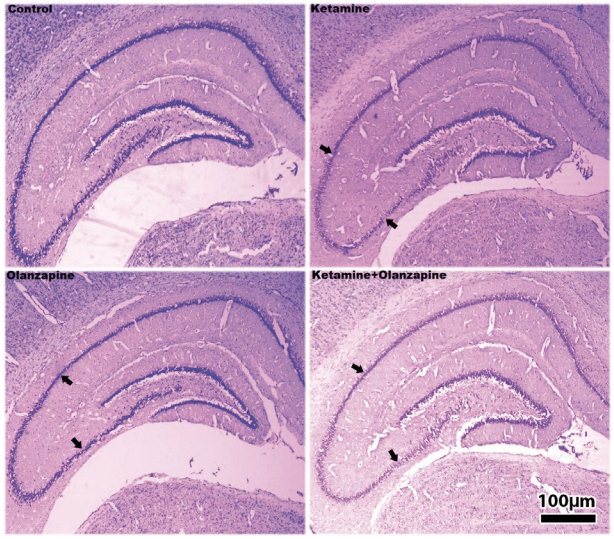

Figure 4 Representative general view of the HC regions with cresyl violet staining of the study groups at high magnification. Arrows show decreased cell density and thinned cell layers of hippocampus regions in experimental groups

Table 4 IGF-1 and IGF-1R Protein Levels in the PFC and HC

Tissue		Control	Ketamine	Olz	Ket+Olz	p
PFC	IGF-1	26.8±1.48	27.6±1.94	69.8±1.48[a]	30.6±2.6[b]	0.000
	IGF-1R	28.2±1.64	12.2±1.92[c]	32.6±3.91	52.6±23.91[d]	0.000
HC	IGF-1	26.8± 1.48[e]	17.4± 3.28	21.8± 1.78	22.6± 1.67	0.000
	IGF-1R	28.2± 1.64	31.4± 3.50	71.6± 3.20[f]	15.6± 4.39[g]	0.000

a post-hoc Tukey control vs olz p=0,000
b post-hoc Tukey control vs ket+olz p=0.031
c post-hoc Tukey control vs ketamine p=0.000
d post-hoc Tukey control vs ket+olz p=0.000
e post-hoc Tukey control vs ketamine p=0.000, control vs olz p=0.011, control vs ket+olz p=0.035
f post-hoc Tukey control vs olz p=0.000
g post-hoc Tukey control vs ket+olz p=0.000

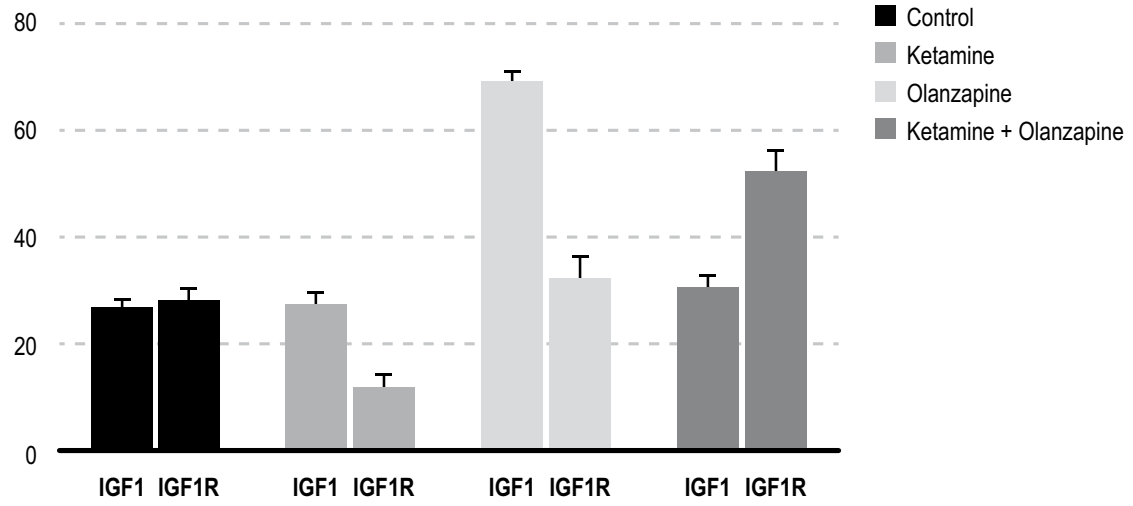

Figure 5 IGF-1 and IGF-1R protein levels in the PFC

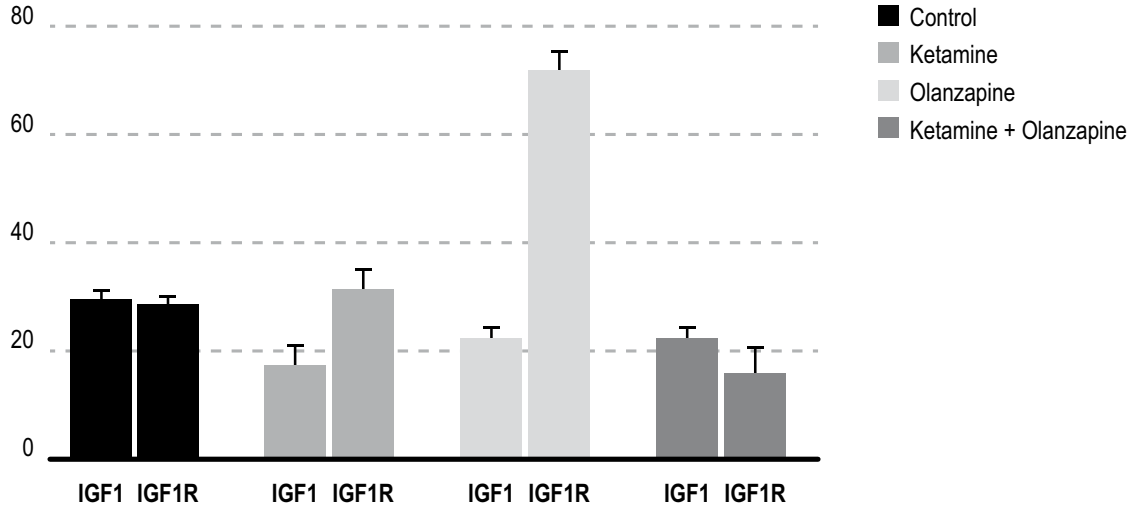

Figure 6 IGF-1 and IGF-1R protein levels in HC

the olanzapine group, no change was observed in volume, but a significant increase was detected in IGF-1 gene expression and protein levels. On the other hand, no significant change was detected in IGF-1R gene expression and protein levels. In the ketamine+olanzapine group, there was no change in volume, but a significant increase was detected in IGF-1 protein, IGF-1R gene expression, and IGF-1R protein levels.

When we examined HC, there was a significant decrease in volume in all three groups compared to the control group. Supporting this reduction in volume, there was a significant decrease in IGF-1 protein levels in all three groups compared to the control group. Although a decrease was detected in IGF-1 gene expression levels in all three groups compared to the control, only the decrease in the ketamine group was statistically significant.

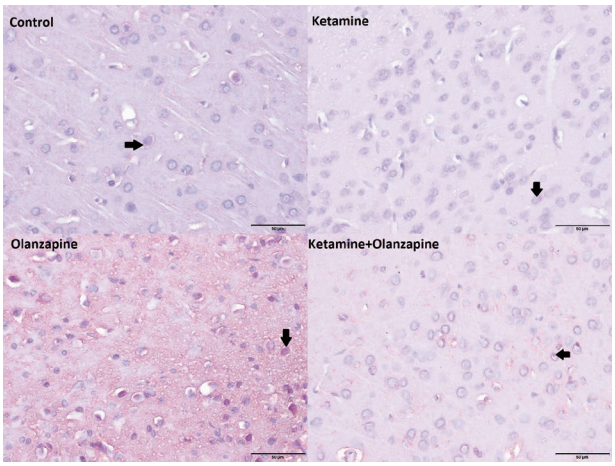

Figure 7 IGF-1 immunohistochemical staining images of PFC groups. Areas of brown staining indicated by arrows indicate neurons with immunopositive staining

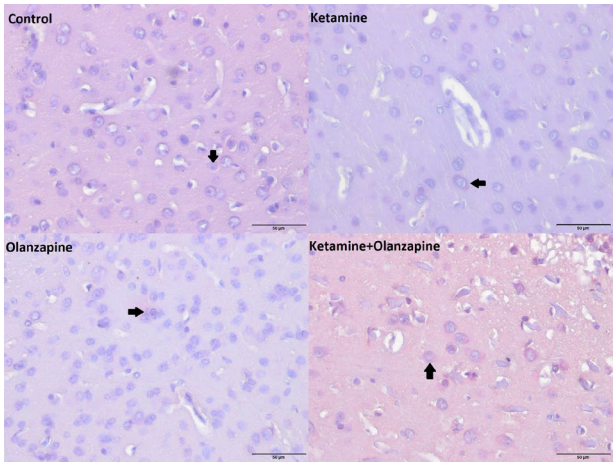

Figure 8 IGF-1R immunohistochemical staining images of PFC groups. Areas of brown staining indicated by arrows indicate neurons with immunopositive staining

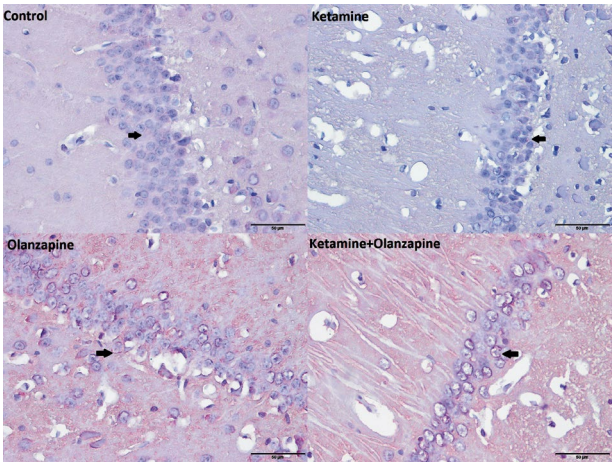

Figure 9 IGF-1 immunohistochemical staining images of HC groups. Areas of brown staining indicated by arrows indicate neurons with immunopositive staining

IGF-1R levels, there was no significant change in IGF-1R gene expression and protein levels in the ketamine group compared to the control group. In contrast, a significant increase was found in IGF-1R gene expression and protein levels in the olanzapine group. There was a significant decrease in IGF-1R gene expression and protein levels in the ketamine+olanzapine group compared to the control.

It is not fully understood which changes are effective in which parts of the brain in the emergence of BD. Volumetric mega-analysis of subcortical structures has shown that BD is associated with increased lateral ventricular size and decreased amygdala, HC, and thalamus volumes (Hibar et al., 2016). In a meta-analysis of hippocampal volume, 6 out of 21 studies found decreased hippocampal volume in patients with BD compared to healthy controls using magnetic resonance imaging and functional magnetic resonance imaging (fMRI) (Otten and Meeter, 2015).In cross-sectional studies of brain structure, it has been reported that there is a decrease in the volume of the brain and prefrontal lobe, and an increase in the volume of the globus pallidus and lateral ventricle throughout life in BD (Arnone et al., 2009). However, not obvious but measurable reductions in cortical thickness were detected in patients with BD in the lateral and medial prefrontal regions, insula, fusiform gyrus, HC, and thalamus volumes (Hibar et al., 2016, 2018; Pezzoli et al., 2018). In our study, we observed that the low volume of PFC and HC, in which we created the bipolar mania model with ketamine, may be related to a detected decrease in the gene expression and protein levels of IGF-1R in the PFC and the gene expression and protein levels of IGF-1 in the HC. Consistent with these findings, one of the studies showed a 10-20% increase in brain weight from late pregnancy with nestin-induced IGF-1 overexpression during embryonic brain development (Popken

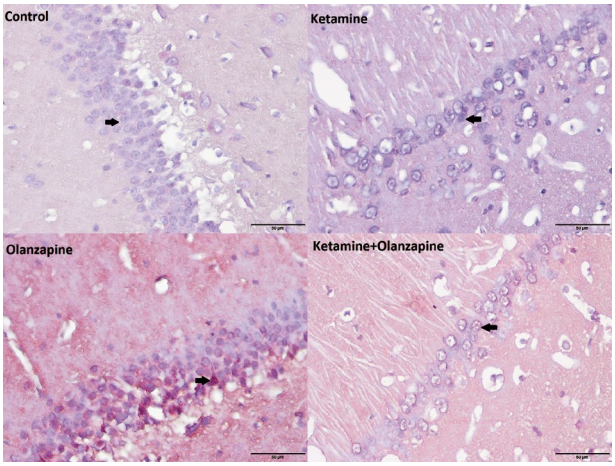

Figure 10 IGF-1R immunohistochemical staining images of HC groups. Areas of brown staining indicated by arrows indicate neurons with immunopositive staining

et al., 2004). In addition, 29-38% and 40% reductions in brain weight have been reported in IGF-1 null mutant mice and mice with deletions of IGF-1R, respectively (Ye et al., 2002; Joseph D'Ercole & Ye, 2008).

The mechanism of action of olanzapine is not known exactly (Joshi et al., 2019), and what changes it causes in the brain is still controversial (Boonstra et al., 2011). Many studies related to the fact that the drug has a neuroprotective effect. It has been suggested that olanzapine has beneficial effects on cognitive impairment and neuropathological changes in the treatment of neurodegenerative diseases (He et al., 2005) and may exhibit some of its therapeutic effects by inducing neurogenesis and/or proliferation of neural progenitors (Chikama et al., 2017; Wakade et al., 2002). In animal experiment studies, it has been reported that olanzapine protects against

methamphetamine-induced high death rates and dopaminergic terminal damage (He et al., 2004), embryonic cortical neurons from glutamate-induced neurotoxicity (Koprivica et al., 2011), and neuronal cells from oxidative stress-induced neurotoxicity (Yang & Lung, 2011). Studies showing the neuroprotective effect of olanzapine have mostly focused on the PFC of the brain. A study conducted with adult rats reported that chronic olanzapine administration increased cell proliferation in the PFC (Kodama et al., 2004). It has been shown that chronic treatment with olanzapine increased the number and survival rate of newly produced cells in the PFC of adult male rats (Wang et al., 2004), reversed 6-hydroxydopamine-induced changes in prefrontal cortical pyramidal cell dendrites (Wang & Deutch, 2008) and have protective effects against asymmetric spine synapses in the PFC after exposure to phencyclidine (Elsworth et al., 2011). These studies show that olanzapine has neuroprotective properties, suggesting that this effect may play a role in treating BD. However, there is no information in the literature about the molecular pathway through which the drug exerts its neuroprotective properties. The IGF-1 and IGF-1R signaling pathway may be one of the pathways in which olanzapine has a neuroprotective effect.

IGF-1 has been proven to assist in impaired neurogenesis, myelination, remyelination, neuromodulation, and synaptogenesis in affective disorders (O'Kusky et al., 2000; Slavich & Irwin, 2014). A decrease in the number of neurons during neurogenesis is detected in IGF-1 and IGF-1R KO mice (Baker et al., 1993; Liu et al., 1993, 2009; Powell-Braxton et al., 1993; Beck et al., 1995; Hurtado-Chong et al., 2009). On the other hand, an increase in the number of neurons and brain size in HC is reported in animals overexpressing IGF-1 (O'Kusky et al., 2000; Ye et al., 2002; Popken et al., 2004; Carlson et al., 2014).

Studies examining the effect of olanzapine on IGF-1 are very few in the literature. In a study with cell culture, olanzapine was shown to increase IGF-1 mRNA expression (Xue et al., 2017). In another study, olanzapine was reported to ameliorate neuropathological changes and increase IGF-1 expression in the frontal cortices of C57BL/6 mice exposed to cuprizone. (Zhang et al., 2014b). Our study detected a significant increase in IGF-1 gene expression and protein levels in rats treated with olanzapine in PFC. Our findings suggest that treating rats with a bipolar mania model with olanzapine (ketamine+olanzapine group) has a neuroprotective effect on the PFC by increasing IGF-1 protein levels and IGF-1R gene expression and protein levels. The absence of a decrease in the volume of the PFC in the olanzapine and ketamine+olanzapine administered groups may be due to these neuroprotective effects.

In addition to studies reporting the neuroprotective effect of olanzapine, there are also studies about its neurodegenerative effect. It has been reported that olanzapine inhibits the growth of glioma cells by inducing autophagy and apoptosis (Wang et al., 2014), and high dose olanzapine causes neurotoxicity in hypothalamic neurons by increasing the production of reactive oxygen species (Boz et al., 2020). In addition, It has been reported that the cortex (frontal, orbitofrontal, medial temporal) was thinner and gray matter volume was reduced after treatment with olanzapine (Gjerde et al., 2018; Molina et al., 2007) and 8-11% reduction in volume was observed in all regions of the brain in animals treated with chronic olanzapine (Dorph-Petersen et al., 2005; Vernon et al., 2011). In our study, there was a significant decrease in HC volume and IGF-1 protein levels in the olanzapine group compared to the control group supports these studies. The increase in IGF-1R gene expression and protein levels in the olanzapine group may have occurred as a compensation mechanism against the decrease in IGF-1 levels. Decreased IGF-1 level may lead to increased IGF-1R level (Bergstedt & Wieloch, 1993). A significant decrease in HC volume and IGF-1 protein levels in the ketamine+olanzapine group seems to support these findings, but a significant decrease in IGF-1R gene expression and protein levels, contrary to the above finding, suggests that there is a drug interaction. However, further studies are needed to clarify this situation.

There are also studies reporting the neuroprotective effect of olanzapine in contrast to its neurotoxic effect on the HC. It has been reported that olanzapine administration did not cause a change in HC volume (Crum et al., 2016) and significantly reduced spatial memory impairment and neuropathological changes caused by okadaic acid (He et al., 2005), reversed kainic acid-induced hippocampal neuronal loss (Csernansky et al., 2006), increased cell proliferation (Kodama et al., 2004) increased density of BrdU-positive cells (Chikama et al., 2017; Wakade et al., 2002) in HC.

One of the most important limitations of our study is that phospho-IGF-1R (phosphorylated IGF-1R) protein levels were not examined. Investigating the autophosphorylation that occurs after binding IGF-1 to its receptor will be useful in elucidating the mechanism of action of olanzapine. The other limitation of our experimental design is the differential use of hemispheres: the right hemisphere was allocated to gene expression and protein assays, whereas the left hemisphere was dedicated to histology. Since both hemispheres were not subjected to all analyses, potential lateralization effects—a factor of documented significance in both clinical (Okada et al., 2023) and preclinical (Pavlova et al., 2023) psychiatric research—could not be ruled out, representing a methodological constraint.

CONCLUSIONS

One of the underlying causes of BD may be changes in IGF-1 and IGF-1R levels in the PFC and HC. Olanzapine, which is used in treating the disease, may have different effects in different brain regions. Our findings indicate that olanzapine may exert a neuroprotective effect by increasing IGF-1 and IGF-1R levels in the PFC, and a neurodegenerative effect by decreasing IGF-1 and IGF-1R levels in the HC. Our study is a pioneering study for the molecular diagnosis and treatment of BD and will be a guide for further studies and clinicians.

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