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Protective effect of 5-HT7 receptor activation against glutamate-induced neurotoxicity in human neuroblastoma SH-SY5Y cells via antioxidative and antiapoptotic pathways☆

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ABSTRACT

Serotonin exerts anti-inflammatory, antioxidant and antiapoptotic effects through 5-HT7 receptors. The present study determined the role of 5-HT7 receptors in glutamate-induced neurotoxicity by using human SH-SY5Y neuroblastoma cells. The cells were pretreated with different concentrations of 5-HT7 receptor agonist LP44 and antagonist SB269970 for 60 min, followed by treatment with glutamate. Cell proliferation was measured using xCELLigence system. Treatment with all the concentrations of LP44 significantly protected the cells from the toxic effects of glutamate after 24, 48 and 72 h. Although 5-HT7 receptor expression was significantly upregulated in glutamate-treated cells, it was downregulated in LP44-pretreated cells. Furthermore, LP44 treatment significantly decreased malondialdehyde levels and increased superoxide dismutase activities and glutathione levels. Moreover, LP44 treatment significantly decreased tumor necrosis factor alpha (TNF-α) levels and inhibited caspase 3 and caspase 9 mRNA expression. In contrast, SB269970 treatment exerted an insignificant effect on oxidative stress, inflammation and apoptosis. These findings suggest that exogenous stimulation of the 5-HT7 receptors may be protective in glutamate-induced neurotoxicity and that 5-HT7 receptor agonists can be used as therapeutic agents for preventing glutamate-induced neurological disorders.

1. Introduction

Glutamate (Glut) is the main excitatory neurotransmitter in the central nervous system (CNS) and an important neurotoxin (Chen et al., 2011; Qian et al., 2011). In patients with brain diseases such as epilepsy, Alzheimer's disease, Parkinson's disease and with trauma and paralysis, stimulant amino acids such as Glut and aspartate are released into the extracellular environment, which induce neuronal damage and degeneration. Glut disrupts intracellular calcium balance by increasing *N*-methyl-D-aspartate (NMDA) receptor expression and induces neurotoxicity by increasing oxidative stress and inflammation (Ankarcrona et al., 1995). Cytochrome c is released to scavenger active oxygen species (ROS) and to partially prevent Glut toxicity (Butterfield, 2003). In the progressive phase of Glut toxicity, cells cannot tolerate increased ROS release, resulting in the development of functional disorders. This results in mitochondrial damage and cellular apoptosis (Liu et al., 2007).

Serotonin, a vasoactive amine is a neurotransmitter that plays an important role in behavioral and psychological events such as pain, appetite, mood and sleep (Mossner and Lesch, 1998). Because of its immunomodulatory activity, Serotonin affects serotonergic receptors (Gordon and Barnes, 2003; Meredith et al., 2005). Serotonergic receptors are classified into seven types based on their structure and exert different biological effects by inducing signal transduction (MaassenVanDenBrink et al., 2008). Among the serotonergic receptors, 5-HT7 receptors have been identified recently and are commonly found in the brain; however, their precise effects are unknown (Hedlund, 2009; Neumaier et al., 2001). 5-HT7 receptor agonists can impact NMDA receptor signaling (Vasefi et al., 2013b). İn study it is shown that 5-HT7 receptor agonism prevents NMDA-induced neurotoxicity in

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hippocampal neurons (Vasefi et al., 2013a). However, no study has investigated the effects of 5-HT7 receptors on Glut-induced toxicity to date.

In recent years, several 5-HT7 receptor agonists and antagonists have been developed and evaluated experimentally. LP44 and SB269970 are potent and selective 5-HT7 receptor agonist and antagonist, respectively and have been widely used in experimental studies. Experimental studies have shown that 5-HT7 receptors modulate the expression of some inflammatory cytokines (Muller et al., 2009). Our previous studies have shown that 5-HT7 receptors play important roles in systemic inflammatory events such as sepsis and acute inflammation in rats (Albayrak et al., 2013; Cadirci et al., 2013). Furthermore, we observed that stimulation of 5-HT7 receptors by agonists improved inflammatory cytokine levels and oxidant parameters (Albayrak et al., 2013). Moreover, serotonin exerts anti-inflammatory and antioxidant effects through 5-HT7 receptors (Cadirci et al., 2013). Another study has reported that 5-HT7 receptors exert an antiapoptotic effect and that 5-HT7 receptor-mediated serotonin-induced extracellular signal-regulated kinase phosphorylation prevents apoptosis by inducing nuclear factor-kappa B (NF-κB) activation (Soga et al., 2007). In addition, 5-HT7 receptor activation prevents the apoptosis of hippocampal neurons in sevoflurane-induced amnesia, thus preventing caspase activation (Zhang et al., 2014).

Therefore, the present study determined the role of 5-HT7 receptors in Glut-induced neurotoxicity by using human SH-SY5Y neuroblastoma cells. We found that 5-HT7 receptors exerted potential neuroprotective effects against Glut-induced toxicity by affecting different pathways, including oxidative stress, inflammation and apoptotic pathways.

2. Materials and methods

2.1. Chemicals and reagents

The selective 5-HT7 receptor antagonist SB269970 (CN:1612, pK (i) = 8.9, chemical formula: $C_{18}H_{28}N_2O_3S{\cdot}HCl$, purity: ≥98% High Performance Liquid Chromatography (HPLC)) and selective 5-HT7 receptor agonist LP44 (CN:2534, Ki = 0.22 nM chemical formula: $C_{27}H_{37}N_3OS{\cdot}HCl$, purity: ≥98% HPLC) were purchased from Tocris Bioscience (Bristol, UNITED KINGDOM). Glut (L-glutamic acid monosodium salt hydrate, G5889, empirical formula: hill notation, molecular weight:169.11 (anhydrous basis)) was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Dulbecco's modified Eagle's medium (DMEM), cell culture medium and reagents such as fetal bovine serum, penicillin/streptomycin, and trypsin were obtained from Gibco (Invitrogen Inc., Grand Island, New York, USA).

2.2. Cell culture

Human SH-SY5Y neuroblastoma cells were purchased from American Type Culture Collection (10801 University Boulevard, Manassas, USA). These cells were grown in DMEM supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, and 10% calf serum at 37 °C in a humidified incubator with an atmosphere of 95% air and 5% CO_2. All the reagents were dissolved in the cell culture medium.

2.3. Proliferation assay

Cell proliferation assay was performed according to a protocol described previously (Palabiyik et al., 2016). Cell proliferation was measured using xCELLigence system (Roche Diagnostics, Indianapolis, Indiana, USA), a label-free technique that dynamically monitors living cells. First, SH-SY5Y cells were seeded in a normal cell culture medium at a density of 5000 cells/well. This study used 80 mM Glut. After 24 h, the cells were pretreated with different concentrations of LP44 and SB269970, which were dissolved in 100 mM DMSO (Dimethyl sulfoxide), for 60 min, followed by treatment with 80 Mm Glut. Cell viability was determined at 24, 48 and 72 h after the Glut treatment by using the xCELLigence system. Data are presented as normalized cell index (CI) ± standard deviation (SD). In xCELLigence System, the parameter termed CI is a measure of the relative change in the electrical impedance at certain frequency (The xCELLigence System monitors the cellular events in real-time by measuring electrical impedance using microelectrodes at the bottom of each cell culture plate well. The RTCA Software calculates the Cell Index (CI) as the relative change in measured impedance to represent cell status). Cell index showed proliferation and viability of the cells.

2.4. Real-time polymerase chain reaction

For mRNA analysis, the cells were seeded in a six-well plate, cultured for 6 h until they formed a monolayer, and incubated with or without Glut, LP44, 5-HT and SB269970.

2.5. Total RNA extraction and cDNA synthesis

The cells were homogenized for 2 min by using TissueLyser II (Qiagen, Hilden, Germany). Total RNA was purified using RNeasy Mini Kit (Qiagen) in QIAcube (Qiagen), according to the manufacturer's instructions. Next, RNA samples were reversetranscribed into complementary DNA (cDNA) by using a high-capacity cDNA reverse-transcription (RT) kit (Applied Biosystems, Foster City, California, USA). RT was performed by treating 10 mL purified RNA with 2 mL 10× RT buffer, 0.8 mL 25× deoxynucleotide mix, 2 mL 10× RT random primers, 1 mL MultiScribe reverse transcriptase, and 4.2 mL diethylpyrocarbonate-treated water at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min in Veriti 96-Well Thermal Cycler (Applied Biosystems). cDNA concentration and quality were assessed using Epoch Spectrophotometer System and Take3 Plate (Biotek, Winooski, Vermont, USA).

2.6. Relative quantification of gene expression

Relative mRNA expression of the genes encoding 5-HT7, caspase 3, and caspase 9 was determined using StepOne Plus real-time polymerase chain reaction (PCR) system (Applied Biosystems) and cDNA synthesized from the SH-SY5Y cell RNA. Expression data obtained for the β-actin gene in each cell group were used as endogenous control. For each cell group, real-time PCR analysis of both caspase 3 and caspase 9 was performed in triplicate by using a 96-well optical plate containing 20 mL reaction mixture comprising 9 mL cDNA (100 ng), 1 mL Primer Perfect Probe mix, and 10 mL QuantiTect Probe PCR master mix (Qiagen). PCR was performed using the following thermal conditions: initial heating at 50°Cfor 2 min and 95 °C for 10 min, followed by 40 cycles of 94 °C for 15 s and 60 °C for 60 s. All data are expressed as fold change in gene expression compared with that in cell groups by using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.7. Biochemical investigations

For biochemical analysis, the cells were seeded in a six-well plate, cultured for 24 h until they formed a monolayer, and incubated with or without Glut, LP44, 5-HT and SB269970.

2.8. Analysis of superoxide dismutase activity and glutathione and malondialdehyde levels

Superoxide dismutase (SOD) activity (Sun et al., 1988) and glutathione (GSH) (Sedlak and Lindsay, 1968) and malondialdehyde (MDA) levels (Ohkawa et al., 1979) in each cell supernatant and standard were measured in duplicate at room temperature by using an enzyme-linked immunosorbent assay (ELISA) reader, according to modified methods. Average absorbance of each sample and standard

was calculated, a standard curve was plotted, and equation for the absorbance of the standard was obtained. Linear concentrations of SOD, GSH, and MDA were calculated using this equation. All data are presented as mean ± SD per milligram of protein.

2.9. Analysis of TNF-α levels

TNF-α levels in each cell supernatant and standard were measured in duplicate by using a highly specific human TNF-α ELISA kit (Invitrogen) and the ELISA reader.

2.10. Determination of protein concentrations

Protein concentrations were determined using Lowry's method and commercial protein standards (total protein kit, TP0300-1KT; Sigma-Aldrich).

2.11. Statistical analysis

Values are expressed as the mean ± S.D. All data were analyzed by One Way ANOVA, post hoc Tukey test. IBM SPSS Statistics 20.0 software program (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analyses. Differences among the groups were determined using Tukey's multiple comparison test and were considered significant at $p < 0.05$ and 95% confidence interval.

3. Results

3.1. Cell proliferation assay

We evaluated the effect of 5-HT7 receptor agonist and antagonist against Glut-induced neurotoxicity in SH-SY5Y cells (Figs. 1 and 2). Our outcome measure is a combination of proliferation and viability. Cell viability was evaluated using an optimum Glut concentration of 80 Mm (Cunha et al., 2016). We observed that treatment with all the concentrations of LP44, especially 10^{-10}, 10^{-9}, 10^{-8} mM LP44, significantly prevented the toxic effect of Glut on the viability of SH-SY5Y cells after 24, 48 and 72 h. (LP44-for 24 h: df:34 (6,28), F:5941- for 48 h: df:34 (6,28), F:32,513 - for 72 h: df:34 (6,28), F: 60,946 Unlike We observed toxic effect due to high dose of LP44, especially 10^{-7}, 10^{-6}, 10^{-5} mM LP44. In contrast, the viability of SB269970-treated cells was not significantly different from that of Glut-treated cells. (SB269970-for 24 h: df:33 (6,27), F:286,885- for 48 h: df:33 (6,27), F:7714 - for 72 h: df:33 (6,27), F: 7703.

3.2. Biochemical investigations

3.2.1. Oxidative stress parameters

We evaluated SOD activity and GSH and MDA levels with ELISA method in SH-SY5Y cells. MDA levels increased in Glut-treated cells compared with that in control cells (Fig. 3). In contrast, SOD activity and GSH levels, which are indicators of the antioxidant system, significantly decreased in Glut-treated cells compared with that in control cells. Treatment with the 5-HT7 receptor agonist LP44 significantly decreased MDA levels and increased SOD activity and GSH levels. However, these parameters were not significantly different between SB269970 and Glut groups. (SOD-df:29(5,24), F:17,147 - GSH-df:29(5,24), F:5998 - MDA-df:29(5,24), F:305,147).

3.2.2. Inflammatory parameters

Next, we analyzed TNF-α levels with ELISA method in SH-SY5Y cells (Fig. 4).TNF-α levels increased significantly in Glut-treated cells compared with that in -control cells. Treatment with the 5-HT7 receptor antagonist SB269970 did not change TNF-α levels compared with Glut treatment. In contrast, treatment with the 5-HT7 receptor agonist LP44 significantly increased TNF-α levels. (df:29(5,24), F:720,288).

3.3. Molecular investigations

3.3.1. Apoptotic parameters

Next, we performed real-time PCR to determine caspase 3 and caspase 9 mRNA expression levels in SH-SY5Y cells (Fig. 4). Caspase 3 and caspase 9 mRNA expression levels were significantly higher Glut-treated cells than in control cells. Treatment of the 5-HT7 agonist LP44 significantly decreased caspase 3 and caspase 9 mRNA expression compared with Glut treatment. In contrast, caspase 3 and caspase 9 mRNA expression levels in SB269970-treated cells were similar to those in Glut-treated cells. (Caspase 3-df:29(5,24), F:186,801 - Caspase 9-df:29(5,24), F:1391,423).

3.3.2. 5-HT7 mRNA expression

We performed real-time PCR to determine 5-HT7 receptor mRNA

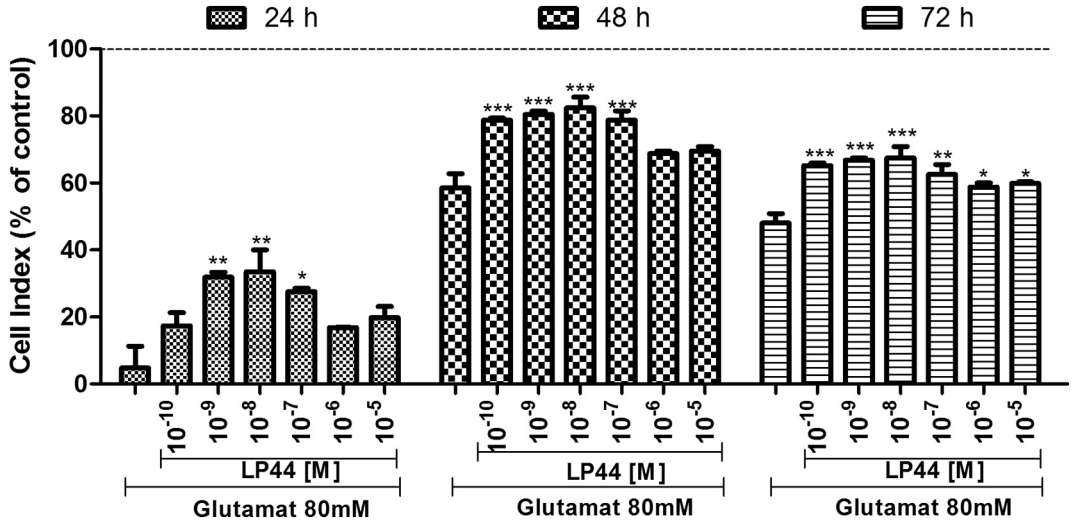

Fig. 1. Remaining cell treated with different concentrations of LP44 in Glutamate-induced neurotoxicity in SH-SY5Y cells. Dashed lines in the figure indicate the amount of control cells as %. Cells were pre-treated with LP44 followed by 80 mM Glutamate. Differences among the groups were determined using the Tukey's multiple comparison test and were considered significant when the p value was less than 0.05 at a 95% confidence interval. All results are given as mean ± SD (Standard Deviation). *: compare with control cells, δ: compare with Glut-treated cells. * $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$, δ $p < 0,05$, δδ $p < 0,01$, δδδ $p < 0,001$.

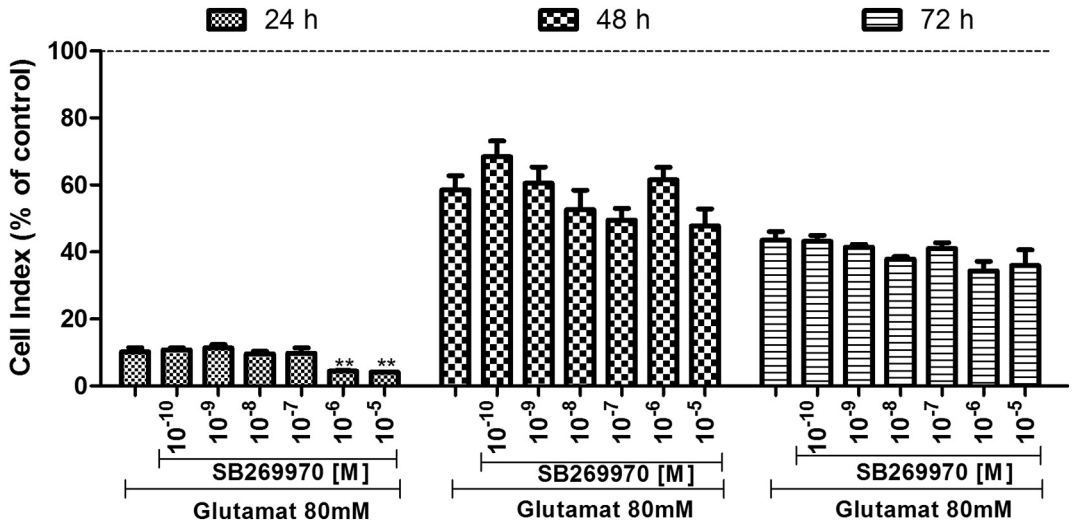

Fig. 2. Remaining cell treated with different concentrations of SB269970 in Glutamate-induced neurotoxicity in SHSY5Y cells. Dashed lines in the figure indicate the amount of control cells as %.

Cells were pre-treated with SB269970 followed by 80 mM Glutamate. Differences among the groups were determined using the Tukey's multiple comparison test and were considered significant when the p value was less than 0.05 at a 95% confidence interval. All results are given as mean ± SD. *: compare with control cells, δ: compare with Glut-treated cells * p < 0,05, ** p < 0,01, *** p < 0,001, δ p < 0,05, δδ p < 0,01, δδδ p < 0,001.

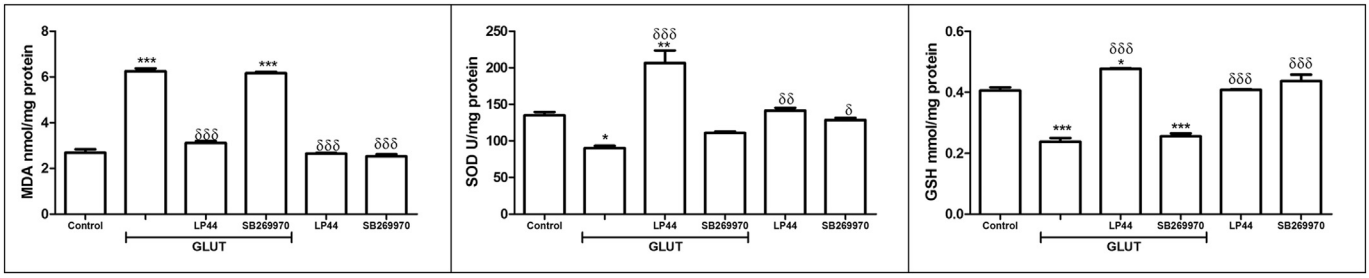

Fig. 3. Effect of 5-HT7 receptors on MDA levels, SOD activity and GSH levels. Cells were pre-treated with 10^{-9} MLP 44 or 10^{-9} M SB269970 by 80 mM Glutamate for 24 h. Differences among the groups were determined using the Tukey's multiple comparison test and were considered significant when the p value was less than 0.05 at a 95% confidence interval. All results are given as mean ± SD. *: compare with control cells, δ: compare with Glut-treated cells. * p < 0,05, ** p < 0,01, *** p < 0,001, δ p < 0,05, δδ p < 0,01, δδδ p < 0,001.

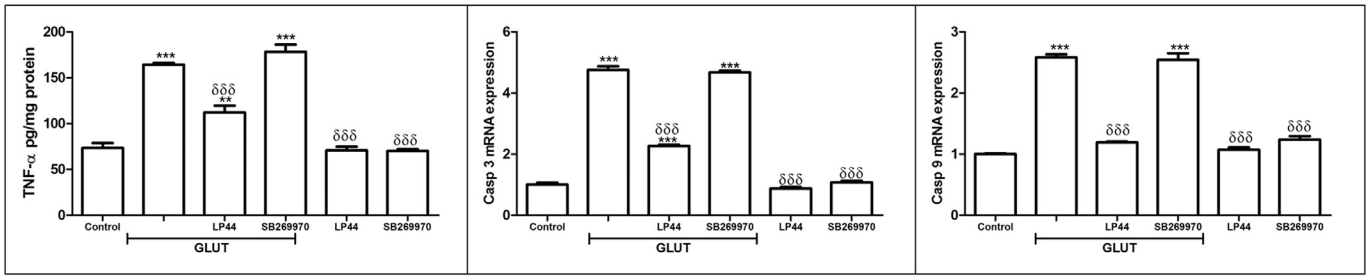

Fig. 4. Effect of 5-HT7 receptors on TNF-a levels, Caspase 3 and Caspase 9 mRNA expressions. Cells were pre-treated with 10^{-9} M LP 44 or 10^{-9} M SB269970 followed by 80 mM Glutamate for 24 h. Differences among the groups were determined using the Tukey's multiple comparison test and were considered significant when the p value was less than 0.05 at a 95% confidence interval. All results are given as mean ± SD. *: compare with control cells, δ: compare with Glut-treated cells * p < 0,05, ** p < 0,01, *** p < 0,001, δ p < 0,05, δδ p < 0,01, δδδ p < 0,001.

expression in SH-SY5Y cells (Fig. 5). 5-HT7 receptor mRNA expression levels were significantly higher Glut-treated cells than in control cells. Treatment with the 5-HT7 agonist LP44 significantly downregulated 5-HT7 receptor mRNA expression compared with Glut treatment. However, treatment with SB269970 did not affect 5-HT7 receptor mRNA expression compared with Glut treatment. (df:29(5,24), F:8086).

4. Discussion

This study examined the hypothesis that 5-HT7 receptors exerted neuroprotective effects in neuronal cultures. The major findings of these study areas follow: (a) 5-HT7 receptor expression is upregulated in SH-SY5Y cells in response to Glut-induced neurotoxicity, (b) the beneficial effects of 5-HT7 receptors may be associated with antioxidant system activation and oxidative stress inhibition, (c) the neuroprotective effects of 5-HT7 receptors are associated with the prevention of Glut-induced TNF-α activation and (d) the beneficial effects of 5-HT7 receptors are associated with caspase 3 and caspase 9 inhibition.

We first determined the cell viability index, an important parameter, by performing the in vitro cell proliferation assay. We observed

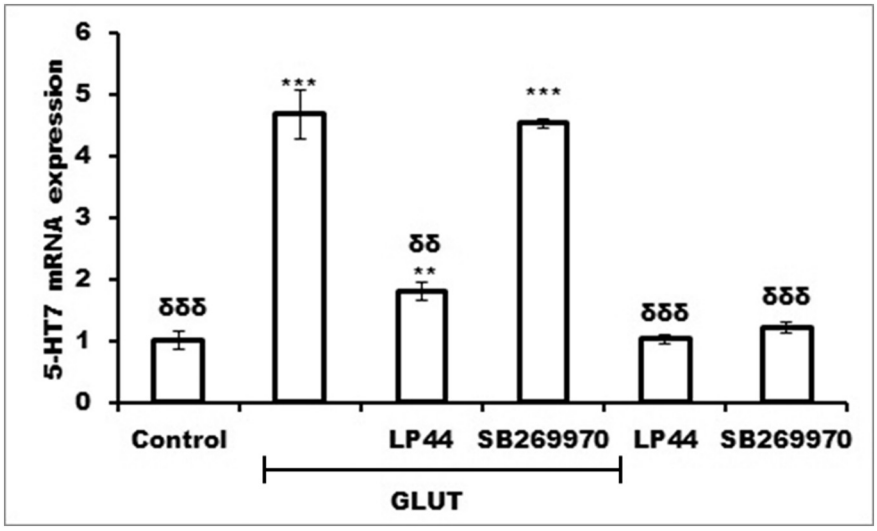

Fig. 5. 5-HT7 mRNA expression levels in toxicity induced by 80 mM Glutamate in SHSY5Y cells for 6 h. Cells were pre-treated with 10^{-9} M LP 44 or 10^{-9} M SB269970 followed by 80 mM Glutamate.
Differences among the groups were determined using the Tukey's multiple comparison test and were considered significant when the p value was less than 0.05 at a 95% confidence interval. All results are given as mean ± SD. *: compare with control cells, δ: compare with Glut-treated cells * $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$, δ $p < 0,05$, δδ $p < 0,01$, δδδ $p < 0,001$.

that the 5-HT7 receptor agonist LP44 exerted potent neuroprotective effects at almost all concentrations. Unlike we observed toxic effect due to high dose and was thought to be due to toxic dose. However, treatment with the 5-HT7 receptor antagonist SB269970 decreased cell viability and did not protect the cells against Glut-induced toxicity.

Several 5-HT7 receptor agonists and antagonists have been used experimentally animal models. LP44 shows high affinity toward 5-HT7 receptors and partially binds to 5-HT1A and 5-HT2A(Gang et al., 2014; Leopoldo et al., 2004).Of the 5-HT7 receptor antagonists identified to date, SB269970 shows the highest affinity and best selectivity (pKi = 8.9) (Pante et al., 2000; Thomas et al., 2000); moreover, it acts as a CNS penetrant. Some studies have investigated the potential utility ofLP44 and SB269970for treating CNS disorders (Gang et al., 2014; Hagan et al., 2000; Monti et al., 2008).We previously examined LP44 and SB269970 and observed that LP44 treatment recovered inflammatory cytokine levels and oxidant parameters by stimulating 5-HT7 receptors (Albayrak et al., 2013; Ayaz et al., 2016; Cadirci et al., 2013).In addition, 5-HT7 receptors modulate the expression of some inflammatory cytokines (Muller et al., 2009). Moreover, LP44 exerts anti-inflammatory and antioxidant effects through 5-HT7 receptors. Vasevi et al. showed that 5-HT7 receptor agonism exerteda neuroprotective effect on hippocampal neurons by activating the PDGFβ receptor, thus inhibiting the NMDA receptors (Vasefi et al., 2013a). In the present study, we observed that 5-HT7 receptor expression was upregulated in Glut-treated cells compared with that in control cells. This upregulation of 5-HT7 receptor expression may be because of a feedback mechanism induced by the increased toxic effect of Glut. LP44 treatment significantly downregulated 5-HT7 receptor expression. Therefore, increased 5-HT7 receptor expression may play an important role in the development of Glut-induced neurotoxicity. To better understand the neuroprotective mechanism of 5-HT7 receptors, we focused on oxidative stress, inflammation and apoptosis and their associated proteins such as GSH, SOD, MDA, TNF-α, caspase 3 and caspase 9.

Glut-induced neurotoxicity is an important model of oxidative stress-mediated neurotoxicity (Chen et al., 2011; Hirata et al., 2011; Kanki et al., 2004). ROS induce the removal of a hydrogen atom from an unsaturated lipid, thus initiating lipid peroxidation. Lipid peroxidation induces considerable changes in cell membrane structure and function and promotes DNA damage, cytotoxicity and eventually cell death. The end-product of lipid peroxidation is MDA, often used as a biomarker to measure oxidative stress levels in organisms (Bessems and Vermeulen, 2001; Fouad and Jresat, 2012).Cellular defenses against oxidative stress involve antioxidant enzymes. Therefore, the use of antioxidants may represent an effective therapeutic strategy for treating oxidative damage (Dawson et al., 1993). SOD and GSH are antioxidant enzymes that eliminates ROS. Reduction in SOD activity in Glut-treated cells may be because of the overproduction of superoxide radical anions. SOD is the main component of the antioxidative system and limit the cytotoxic effects of toxic free radicals (Fink, 2002). Elevated levels of extracellular Glut inhibit cysteine uptake by inhibiting the antiporter system. Inhibited uptake of cysteine, a precursor of GSH, markedly decreases intracellular GSH levels, thereby inducing oxidative stress within cells (Conrad and Sato, 2012; Ma et al., 2012). Many Glut toxicity studies have reported reduced antioxidant levels and increased oxidative stress (Kim et al., 2013; Nampoothiri et al., 2014). In the present study, Glut treatment significantly increased oxidative stress and reduced antioxidant balance. However, 5-HT7 receptor agonist pretreatment significantly reduced Glut-induced MDA levels and increased SOD activity and GSH levels. In contrast, 5-HT7 receptor antagonist pretreatment exerted an insignificant effect on oxidative stress. Previous studies have reported that 5-HT7 receptor agonists exert antioxidant effects by reducing MDA levels and by increasing SOD activity and GSH levels (Albayrak et al., 2013; Cadirci et al., 2013). Agonist administration decreased oxidative stress in SH-SY5Y cells, prompting us to think that stimulation of 5-HT7 receptors protected SH-SY5Y cells by reducing oxidative stress. Herefore, stimulation of 5-HT7 receptors might be a potential therapeutic target for prevention of Glut-induced neurotoxicity.

We examined the association between the decrease in oxidative stress through 5-HT7 receptors and TNF-α levels. Oxidative stress is strongly associated with cytokine levels, especially TNF-α levels (Fischer and Maier, 2015).Moreover, proinflammatory cytokines are the key mediators of neuroinflammation (Chaparro-Huerta et al., 2008; Kigerl et al., 2009) and play an important role in neuronal response to neuronal injury (Mizuno et al., 2008). Strong evidence suggests that Glut-stimulated proinflammatory cytokines are associated with the pathophysiology and progression of neuronal diseases (Hagan et al., 2000). Increased TNF-α levels lead to oxidative stress. Previous studies have shown that TNF-α levels and oxidative stress decrease parallelly during the healing process (Liu et al., 2011). We observed that TNF-α levels increased significantly in Glut-treated SH-SY5Y cells. However, pretreatment with the 5-HT7 receptor agonist LP44 significantly prevented the Glut-induced increase in TNF-α levels. Moreover, we previously found that 5-HT7 receptor agonism reduced both oxidative stress and inflammation. Collectively, these results indicate that 5-HT7 receptors exert antioxidant and anti-inflammatory effects and prevent Glut-induced neuronal degeneration.

Finally, we evaluated the levels of apoptotic proteins, which induce neuronal cell death in response to Glut toxicity. Glut treatment

activates caspase protein cascades, leading to neuronal cell death through apoptosis. Caspases are the chief proteins that regulate apoptotic pathways, which trigger mitochondrial permeability transition and cytochrome *c* release, thus activating initiator and effector caspases (caspase 3 and caspase 9) (Wang et al., 2011). Moreover, ROS promote apoptosis by targeting caspase 3 and caspase 9 activity (Kirkland et al., 2010). Caspase 9 initiates apoptosis and cleaves pro-caspase 3. Caspase 3 acts as an apoptotic executor in the later phase of apoptosis, ultimately leading to cell death. Moreover, as mentioned previously, there is a strong correlation between increased TNF-α levels and caspase 3 and caspase 9 levels. Decreased caspase levels inhibit TNF-α expression, thus ameliorate oxidative stress.

Although the functions of 5-HT7 receptors need to be clarified further, it has been suggested that these receptors regulate apoptosis. A previous study showed that 5-HTinduced ERK phosphorylation and NF-κB activation by stimulating 5-HT7receptors (Soga et al., 2007). Activation of these signaling pathways by 5-HT may exert an antiapoptotic effect. Moreover, Zhang et al. showed that 5-HT7 receptor agonists exerted an antiapoptotic effect on sevoflurane-induced amnesia in hippocampal neurons by inhibiting caspase 3 (Zhang et al., 2014).

Our results showed that caspase 3 and caspase 9 expression increased in Glut-treated cells compared with that in control cells. However, LP44 treatment significantly decreased caspase 3 and caspase 9 expression in SH-SY5Y cells. In contrast, 5-HT7 receptor antagonist treatment exerted an insignificant effect on caspase 3 and caspase 9 expression.

These findings indicate that 5-HT7 receptors play a critical role in Glut-induced neurotoxicity. 5-HT7 receptor activation by selective agonists prevents oxidative stress, inhibits proinflammatory cytokines and exerts an antiapoptotic effect by decreasing caspase3and caspase 9 gene expression. Our results can be used as a basis for performing further clinical studies because 5-HT7 receptors may be useful for preventing neurodegenerative disorders.

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The authors declare that they do not have any conflict of interest related to this study.

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