Original Article

Duloxetine by Modulating the Akt/GSK3 Signaling Pathways Has Neuroprotective Effects against Methamphetamine-Induced Neurodegeneration and Cognition Impairment in Rats

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Received: 29 May 2017
Revised: 5 September 2017
Accepted: 15 October 2017

What's Known

• Methamphetamine abuse can cause neurodegenerative disorders and neurobehavioral changes. Protective properties of duloxetine against neurobehavioral and neurodegenerative disorders were reported by previous investigations.

What's New

• Current study indicated the protective role of duloxetine in the amelioration of methamphetamine-induced anxiety, depression, motor activity disturbance, and cognition impairment.

Abstract

Background: The neuroprotective effects of duloxetine, as an antidepressant agent, and the neurodegenerative effects of methamphetamine have been shown in previous studies. Nonetheless, their exact neurochemical and behavioral effects are still unclear. In the current study, we sought to clarify the molecular mechanisms involved in the protective effects of duloxetine against methamphetamine-induced neurodegeneration.

Methods: Forty adult male rats were divided randomly into 5 groups. Group 1 was the negative control and received normal saline, Group 2 was the positive control and received methamphetamine, and Groups 3, 4, and 5 were concurrently treated with methamphetamine (10 mg/kg) and duloxetine (5, 10, and 15 mg/kg, respectively). All the treatments were continued for 21 days. Between days 17 and 21, the Morris Water Maze (MWM) was used to assess learning and memory in the treated groups. On day 22, the hippocampus was isolated from each rat and oxidative, antioxidant, and inflammatory factors were measured. Additionally, the expression levels of the total and phosphorylated forms of the Akt and GSK3 proteins were evaluated via the ELISA method.

Results: Duloxetine in all the administered doses ameliorated the effects of the methamphetamine-induced cognition impairment in the MWM. The chronic abuse of methamphetamine increased malondialdehyde, tumor necrosis factor-α, and interleukin-1β, while it decreased superoxide dismutase, glutathione peroxidase, and glutathione reductase activities. Duloxetine not only prevented these malicious effects of methamphetamine but also activated the expression of Akt (both forms) and inhibited the expression of GSK3 (both forms) in the methamphetamine-treated rats.

Conclusion: We conclude that the Akt/GSK3 signaling pathways might have a critical role in the protective effects of duloxetine against methamphetamine-induced neurodegeneration and cognition impairment.

Introduction

Duloxetine is an antidepressant of serotonin–norepinephrine reuptake inhibitors (SNRIs) and is used to treat depression and anxiety and to enhance cognition.[1,2] Some recent studies have revealed that this agent, because of its effects on the reuptake of both serotonin and norepinephrine, can be effective as a sedative and anxiolytic agent.[1,2] These studies have suggested that since duloxetine has both anxiolytic and antidepressant effects, it can be used to treat the abuse of amphetamine and other psychostimulants and to modulate the cognitive and neurodegenerative effects of methamphetamine abuse.[1,2] Further, this agent has neuroprotective and anti-inflammatory effects and can act against some neurodegenerative situations such as ischemia.[3] Duloxetine can also be effective in the treatment of the abuse of other drugs such as alcohol.[4,5] The neuroprotective effects of duloxetine and its role in the inhibition of oxidative stress, inflammation, and apoptosis have been reported in several investigations; the exact molecular mechanisms of these effects, however, have hitherto remained ambiguous.[3, 6]

Methamphetamine is a neurostimulant with an increased rate of abuse in recent years.[7] The mechanisms of the biochemical and behavioral consequences of the chronic abuse of methamphetamine are still far from clear in adults and children.[8-11] The mechanism of methamphetamine action is to increase the release of dopamine, norepinephrine, and to a lesser extent serotonin into synaptic terminals, causing the hyperstimulation of receptors in the acute phase and the downregulation of receptors in the chronic phase.[8-11] Methamphetamine functionally and pharmacologically is similar to cocaine and this similarity creates a high potential for abuse and addiction.[7] The chronic abuse of methamphetamine and also its withdrawal can induce behavioral changes such as cognition (learning and memory) impairment in human and experimental models.[8-11] Experimental studies have confirmed the potential of methamphetamine in inducing neurodegeneration in some areas of the brain such as the hippocampus, which is responsible for cognition impairment.[9] Research has also confirmed methamphetamine-induced oxidative stress, inflammation, and apoptosis in brain areas such as the hippocampus; nevertheless, what has thus far remained unclear is the molecular aspects and the signaling pathways involved.[12, 13]

Many previous investigations have indicated that phosphatidylinositol 3-kinase can activate protein kinase B (Akt) in brain cells. This activation inhibits glycogen synthase kinase 3 (GSK3), which is involved in neurodegeneration, and protects cells from its neurodegenerative effects.[14,15] Earlier research has also demonstrated the role of the Akt/GSK3 signaling pathways in cognitive activity.[14]

Because of the importance of the Akt/GSK3 signaling pathways in the modulation of neuroprotection and cognition performance, we designed the present study to assess the role of these pathways in conferring the neuroprotective effects of duloxetine against methamphetamine-induced neurodegeneration and alterations in cognitive activities. We hope that the results of the current study will confer a better understanding of amphetamine toxicity and the mechanisms involved.

Materials and Methods

Animals: Forty adult male Wistar rats, weighing between 250 and 300 g, were obtained from the Animal House of Iran University of Medical Sciences. They were kept under a controlled temperature (22±0.5 °C) with 12-hour light/dark cycles and had free access to food and water. Our experiments were undertaken in Iran University of Medical Sciences (Tehran, Iran) in 2017, and our experimental protocol, which adhered to the guidelines on animal ethics and welfare, was approved by the Ethics Committee of Iran University of Medical Sciences.[16]

Drugs: Methamphetamine and duloxetine were purchased from Sigma-Aldrich Co. (St. Louis, Missouri, USA), and they were freshly prepared just before use. Methamphetamine was dissolved in normal saline, and duloxetine was dissolved in warmed normal saline. The exact doses of methamphetamine and duloxetine were calculated based on the animals' weight; these amounts were dissolved in 0.2 mL/rat as the volume of injection for each rat.

Experimental design: Group 1, the negative control, was administrated with normal saline (0.2 mL/rat, i.p.) for 21 days and Group 2, the positive control, received methamphetamine (10 mg/kg, i.p., 0.2 mL/rat) for 21 days. Groups 3, 4, and 5 were treated concurrently with methamphetamine (10 mg/kg, i.p., 0.2 mL/rat) and duloxetine (5, 10, and 15 mg/kg, i.p., 0.2 mL/rat) respectively, for 21 days.

Between days 17 and 21, the Morris Water Maze (MWM) was used to evaluate learning and spatial memory in all the treated animals. The effects of duloxetine against methamphetamine-induced neurodegeneration and the role of the Akt/GSK3 signaling pathways were studied by anesthetizing all the rats on day 22 through the administration of

50 mg/kg of thiopental. Afterward, the animals' brain tissues were removed and the hippocampus was isolated from each rat according to the guidelines presented in previous studies.[17] The hippocampus from the right hemisphere was used for the evaluation of oxidative stress and inflammation biomarkers, whereas the left hemisphere's hippocampus was used for the evaluation of the expression of the Akt and GSK3 proteins.

Behavioral Studies

MWM task: The MWM, as the standard behavioral test for the evaluation of cognition and spatial memory, was performed based on previous studies.[18] This test evaluates the time of escape latency (characterized by time to find a hidden platform), traveled distance (measured as the distance each animal covers to reach the hidden platform), and percentage of the presence of the animals in the target quarter.[18]

Molecular Study

Determination of oxidative stress parameters: The levels of lipid peroxidation, malondialdehyde (MDA) production, interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) as well as the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) were measured as described previously by standard protocols.[19, 20]

Measurement of the expression of the proteins: The immunoreactivity of Akt (total and phosphorylated) and GSK3 (total and phosphorylated) was studied in the isolated hippocampus using an ELISA commercial kit (MyBioSource, San Diego, USA) according to previous studies.[14, 21]

Statistical Analysis

The data were analyzed using GraphPad PRISM, version 6 (2016). First, the normality of the continuous variables (behavioral and molecular parameters) was assessed using the Kolmogorov–Smirnov test. Based on this test, all the variables were normally distributed. All the data were described as means±standard errors of the mean (SEMs), and the differences between the treatment groups were evaluated using one-way ANOVA with the Bonferroni posttest for group-by-group comparisons. The results were considered significant at a P level less than 0.05.

Results

Duloxetine Improved Spatial Memory in Methamphetamine-Induced Cognition Impairment

The parameters of escape latency and traveled distance were increased during 4 days of training in the MWM for the groups under treatment with methamphetamine (10 mg/kg); the difference with the negative control group constituted statistical significance (P≤0.001 for escape latency and P=0.007 for the traveled distance) (figures 1 and 2). The swimming speed was not altered during the training trials in the control and methamphetamine-treated groups (P=0.95) (figure 3). Moreover, the presence (%) in the target quarter was reduced among the animals receiving methamphetamine (10 mg/kg) (P≤0.001) (figure 4). Duloxetine in all the administered doses ameliorated the methamphetamine-induced reduction in escape latency (P≤0.001) and the percentage of the presence of the animals in the target quarter
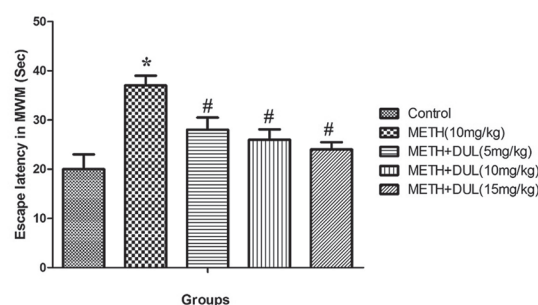

Figure 1: Average of escape latency in the negative control group, the group under treatment with 10 mg/kg of methamphetamine (positive control), and the groups under treatment with methamphetamine in combination with duloxetine (5, 10, and 15 mg/kg) across all the training days using the Morris Water Maze (MWM) in the rats. All the data are expressed as means±SEMs (n=8). METH: Methamphetamine; DUL: Duloxetine; *Shows the level of significance (P≤0.001) in comparison to the negative control group. #Shows the level of significance (P≤0.001) in comparison to the positive control group (10 mg/kg of methamphetamine).
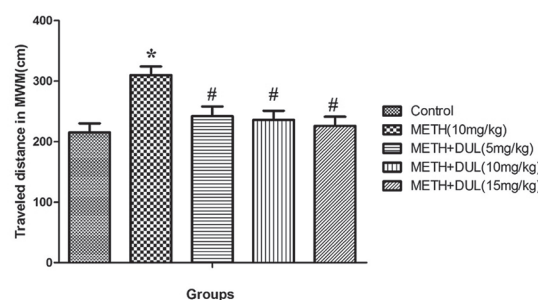

Figure 2: Traveled distance in the negative control group, the group under treatment with 10 mg/kg of methamphetamine (positive control), and the groups under treatment with methamphetamine in combination with duloxetine (5, 10, and 15 mg/kg) across all the training days using the Morris Water Maze (MWM) in the rats. All the data are expressed as means±SEMs (n=8). METH: Methamphetamine; DUL: Duloxetine; *Shows the level of significance (P=0.007) in comparison to the negative control group. #Shows the level of significance (P=0.007) in comparison to the positive control group (10 mg/kg of methamphetamine).
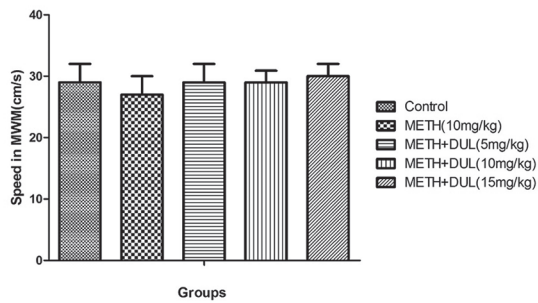
Figure 3: Swimming speed in the negative control group, the group under treatment with 10 mg/kg of methamphetamine (positive control), and the groups under treatment with methamphetamine in combination with duloxetine (5, 10, and 15 mg/kg) across all the training days using the Morris Water Maze (MWM) in the rats. All the data are expressed as means±SEMs (n=8). METH: Methamphetamine; DUL: Duloxetine;
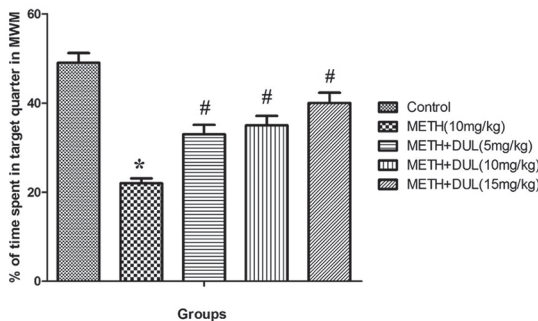

Figure 4: Percentages of the time spent in the target quarter in the probe trial in the negative control group, the group under treatment with 10 mg/kg of methamphetamine (positive control), and the groups under treatment with methamphetamine in combination with duloxetine (5, 10, and 15 mg/kg) across all the training days using the Morris Water Maze (MWM) in the rats. All the data are expressed as means±SEMs (n=8). METH: Methamphetamine; DUL: Duloxetine; *Shows the level of significance (P≤0.001) in comparison to the negative control group. #Shows the level of significance (P≤0.001) in comparison to the positive control group (10 mg/kg of methamphetamine).

(P≤0.001) and increased the traveled distance (P=0.007) as compared to the methamphetamine (10 mg/kg)-treated groups (figures 1, 2, and 4). The swimming speed was not altered during the training trials in the methamphetamine-treated groups in comparison with the duloxetine-treated groups (P=0.95) (figure 3).

Duloxetine Prevented Methamphetamine-Induced Oxidative Stress

Methamphetamine administration significantly increased lipid peroxidation as indicated by elevated mitochondrial MDA levels. It also decreased the activities of SOD, GPx, and GR when compared to the negative control (P≤0.001 for MDA, P=0.013 for SOD, P=0.025 for GPx, and P=0.024 for GR) (table 1). Conversely, the various doses of duloxetine (5, 10, and 15 mg/kg) reduced the methamphetamine-induced rise in the MDA level and ameliorated the methamphetamine-induced decrease in SOD, GPx, and GR activities when compared to the positive controls (P≤0.001 for MDA, P=0.013 for SOD, P=0.025 for GPx, and P=0.024 for GR) (table 1).

Duloxetine Prevented a Methamphetamine-Induced Rise in Inflammatory Biomarkers

The animals in the methamphetamine-treated groups demonstrated significant elevations in IL-1β and TNF-α levels as compared to the negative control group (P=0.008 for IL-1β and P≤0.001 for TNF-α) (table 1). Conversely, the high doses of duloxetine (10 and 15 mg/kg) prevented a methamphetamine-induced rise in pro-inflammatory biomarkers when compared to the positive controls (P=0.008 for IL-1β and P≤0.001 for TNF-α) (table 1).

Duloxetine Inhibited Methamphetamine-Induced Alterations in the Expressions of Both Forms of the Akt and GSK3 Proteins

Methamphetamine (10 mg/kg) treatment markedly reduced the relative protein expression/level of Akt (total and phosphorylated) and GSK3 (total and phosphorylated) in the rats' hippocampus in comparison to the negative control group (P=0.009 for total Akt, P=0.008 for phosphorylated Akt, P≤0.001 for total GSK3, and P=0.002 for phosphorylated GSK3) (figures 5, 6, 7, and 8). Conversely, the high doses of duloxetine (10 and 15 mg/kg) treatment significantly improved the protein expression/level of Akt (total and phosphorylated) and GSK3 (total and phosphorylated) in the methamphetamine-treated animals when compared to the methamphetamine-only-treated group (P=0.009 for total Akt, P=0.008 for phosphorylated Akt, P≤0.001 for total GSK3, and P=0.002 for phosphorylated GSK3) (figures 5, 6, 7, and 8).

Discussion

The current study demonstrated that multiple doses of duloxetine were able to modulate the methamphetamine-induced oxidative stress, inflammation, and cognition (learning and memory) impairment. According to our findings, duloxetine modulated the Akt/GSK3 signaling pathways and thus ameliorated the behavioral and molecular changes induced by methamphetamine administration.

Methamphetamine is a neural stimulant with an increasing abuse rate among youngsters, due to its similarity to cocaine, in recent

Table 1: Effects of the various doses of duloxetine on the alterations of oxidative stress and inflammatory biomarkers in the mitochondria of the rats treated with methamphetamine (10 mg/kg/d)

Group	MDA (nmol/mg of protein)	SOD (U/mL/mg protein)	GPx (U/mL/mg protein)	GR (U/mL/mg protein)	TNF-α (ng/mL)	IL-1β (ng/mL)
Control	6.1±0.9	66.2±4.1	71.2±6.2	51.1±4.1	56.4±6.1	50.3±4.2
Meth (10mg/kg)	20.4±1.4[a]	30.3±3.1[a]	36.2±6.4[a]	22.1±3.2	100.5±8.6[a]	92.6±8.1[a]
Meth (10mg/kg) + Duloxetine (5 mg/kg)	13±1.6[b]	45.4±5.2[b]	49.3±7.1[b]	33.2±5.1[b]	90.5±5.9[a]	88.1±9.1[a]
Meth (10mg/kg) + Duloxetine (10 mg/kg)	11±2[b]	51.4±4.3[b]	59.1±5.2[b]	41.1±5.2[b]	68.5±6.5[b]	64.6±2.9[b]
Meth (10 mg/kg) + Duloxetine (15 mg/kg)	10±2.1[b]	58.2±5.4[b]	66.2±6.1[b]	48.4±4.1[b]	60.5±5.5[b]	61.8±2.2[b]

[a]shows the level of significance with P≤0.001(MDA level), P=0.013 (for SOD), P=0.025 (for GPx), P=0.024 (for GR), P=0.008 (for IL-1β), and P≤0.001 (for TNF-α) vs. the negative control groups; [b]shows the level of significance with P≤0.001(MDA level), P=0.013 (for SOD), P=0.025 (for GPx), P=0.024 (for GR), P=0.008 (for IL-1β), and P≤0.001 (for TNF-α) vs. the positive control group (methamphetamine only). MDA: Malondialdehyde; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; Meth: Methamphetamine
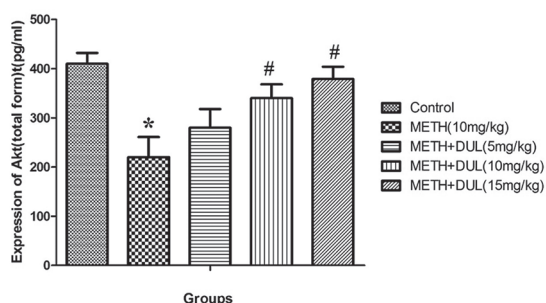

Figure 5: Alterations in the expression/level (ELISA) of Akt (total form) in the hippocampus in the negative control group, the group under treatment with 10 mg/kg of methamphetamine (positive control), and the groups under treatment with methamphetamine in combination with duloxetine (5, 10, and 15 mg/kg). All the data are expressed as means±SEMs (n=8). METH: Methamphetamine; DUL: Duloxetine; *Shows the level of significance (P=0.009) in comparison to the negative control group. #Shows the level of significance (P=0.009) in comparison to the positive control group (10 mg/kg of methamphetamine).
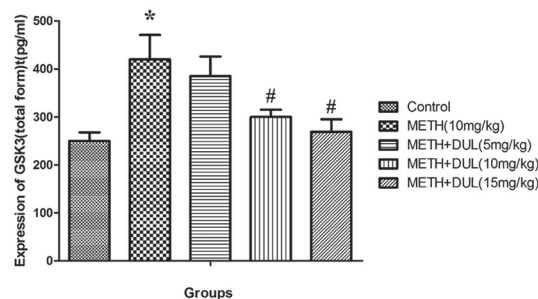

Figure 7: Alterations in the expression/level (ELISA) of GSK3 (total form) in the hippocampus in the negative control group, the group under treatment with 10 mg/kg of methamphetamine (positive control), and the groups under treatment with methamphetamine in combination with duloxetine (5, 10, and 15 mg/kg). All the data are expressed as means±SEMs (n=8). METH: Methamphetamine; DUL: Duloxetine; *Shows the level of significance (P≤0.001) in comparison to the negative control group. #Shows the level of significance (P≤0.001) in comparison to the positive control group (10 mg/kg of methamphetamine).

Figure 6: Alterations in the expression/level (ELISA) of Akt (phosphorylated form) in the hippocampus in the negative control group, the group under treatment with 10 mg/kg of methamphetamine (positive control), and the groups under treatment with methamphetamine in combination with duloxetine (5, 10, and 15 mg/kg). All the data are expressed as means±SEMs (n=8). METH: Methamphetamine; DUL: Duloxetine; *Shows the level of significance (P=0.008) in comparison to the negative control group. #Shows the level of significance (P=0.008) in comparison to the positive control group (10 mg/kg of methamphetamine).
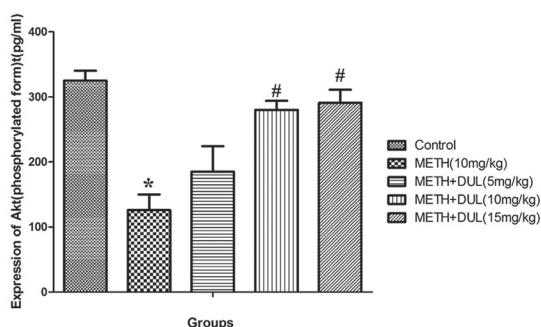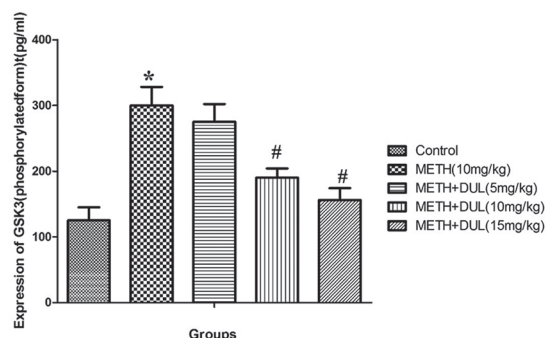

Figure 8: Alterations in the expression/level (ELISA) of GSK (phosphorylated form) in the hippocampus in the negative control group, the group under treatment with 10 mg/kg of methamphetamine (positive control), and the groups under treatment with methamphetamine in combination with duloxetine (5, 10, and 15 mg/kg). All the data are expressed as means±SEMs (n=8). METH: Methamphetamine; DUL: Duloxetine; *Shows the level of significance (P=0.002) in comparison to the negative control group. #Shows the level of significance (P=0.002) in comparison to the positive control group (10 mg/kg of methamphetamine).

years.[8, 22] Methamphetamine augments the release of dopamine and norepinephrine into the synaptic cleft, but the neurobehavioral and neurochemical consequences of its chronic abuse remain unclear.[22] Duloxetine is an SNRI antidepressant used primarily for the treatment of depression, general anxiety disorder, social phobia, panic disorder, and vasomotor symptoms.[23,24] Duloxetine can be effective in the treatment of neurobehavioral and neurochemical disorders related to various drug abuse and their withdrawal syndromes; be that as it may, its exact mechanism has hitherto remained uncertain.[2,22] We found that the chronic administration of methamphetamine at a dose of 10 mg/kg increased escape latency and the traveled distance in the MWM in terms of learning time. Additionally, methamphetamine administration (10 mg/kg) decreased learning activity and also on the probe test day, it lessened the percentage of the presence of the animals in the target quarter in the MWM, which is associated with malicious effects on memory performance. These data suggest that the long-term administration of methamphetamine can attenuate spatial memory in rodents and also confirm the results of the previous investigations having reported that the chronic administration of methamphetamine or the withdrawal of this neurostimulant reduces cognitive function in rats.[25-27] Methamphetamine-like compounds lead to the release of dopamine, serotonin, and noradrenaline in the brain, triggering the downregulation of the mentioned neurotransmitter receptors. The consequence of this phenomenon is cognition impairment during the abuse or withdrawal of this agent.[25-27] According to our results, duloxetine at all the administered doses was able to alter the methamphetamine-induced learning and memory impairment. Many previous studies have indicated that SNRIs such as duloxetine have significant effects on learning and memory and can exert positive effects on cognitive activity.[6,22,28] In our molecular findings, methamphetamine (10 mg/kg) altered oxidative stress and neuroinflammation. We observed that whereas methamphetamine decreased SOD, GPx, and GR activities, it increased the MDA level (a marker of lipid peroxidation) in the rat hippocampus. Previous studies have indicated that the chronic administration of methamphetamine begets mitochondrial dysfunction and alteration in respiratory chain enzymes in the brain cells of rodents. These studies have suggested that methamphetamine can induce oxidative stress in the brain of rats.[29,30] Nevertheless, the exact mechanism of the action of methamphetamine

in this regard still remains undetermined.[30] The results of the current study chime in with those of the previous studies having reported methamphetamine-induced lipid peroxidation in brain cells.[31,32] Some novel reports have shown that methamphetamine consumption inhibits antioxidant activity in multiple cells, and these effects create methamphetamine-induced degenerative impacts on body cells such as those in the brain, liver, and heart.[32] Previous studies have reported that methamphetamine abuse diminishes antioxidant defense, which may result in neurodegeneration.[31,32] In accordance with previous studies, treatment by duloxetine in the current study was found to be effective in reversing this methamphetamine-induced increase in the MDA level and reversing the reduction in SOD, GPx, and GR activities in the hippocampal tissues.[3,33,34] The role of SNRIs such as duloxetine in the activation of antioxidant defense and the increase in the activities of antioxidant enzymes has been previously reported by multiple studies.[34,35] All of these investigations have reported that duloxetine and other similar compounds can activate mitochondrial antioxidant enzymes and by this type of activation can be involved in neuroprotection against some neurotoxic agents such as morphine and amphetamine- type stimulants.[34,35] Our results demonstrated that the inflammatory biomarkers in the hippocampus were increased by methamphetamine administration at a dose of 10 mg/kg. Previous similar studies have indicated that the chronic administration of methamphetamine increases pro-inflammatory markers such as TNF-α and IL-1β, which is consistent with our findings, although the signaling mechanism of these effects has yet to be elucidated.[31] Furthermore, we found that duloxetine inhibited methamphetamine-induced inflammation; this finding is concordant with previous studies.[34,35] The anti-inflammatory role of duloxetine and other antidepressants has been shown already, and this property is the reason for its effectiveness in autoimmune disorders[34,35] and neuroprotective effects against neurotoxic agent-induced inflammation.[4, 34, 35] As was noted above, duloxetine ameliorated the methamphetamine-induced cognition impairment, oxidative stress, and inflammation in the hippocampal cells of our rats; this finding to some extent confirms the results of previous studies on the neuroprotective effects of duloxetine.[4, 34, 35] Nonetheless, to define the mechanism of its neuroprotective action, we sought to evaluate the molecular basis and the possible signaling pathways involved in cognitive activity and neurodevelopment. Thus,

we evaluated the Akt/GSK3 signaling pathways and found that methamphetamine decreased the Akt protein level/expression in both total and phosphorylated forms, while it increased GSK3 in both forms. These data are in line with the previous investigations having shown that methamphetamine-type stimulants inhibit the phosphorylation of Akt in brain cells and that this inhibition of Akt leads to the activation and phosphorylation of GSK3.[36] According to the present data, by the activation of GSK3, some neurodegenerative events will occur in brain cells and some neurobehavioral disorders such as cognition impairment can be related to the inhibition of Akt and the activation of GSK3, involved in neurodegeneration.[15] In the present study, duloxetine ameliorated the methamphetamine-induced decrease in the Akt protein level/expression in both total and phosphorylated forms, while it decreased GSK3 in both forms in the methamphetamine-treated rats.[33, 37] It has been shown by many previous studies that antidepressant effects against neurodegeneration are mediated by the modulation of the Akt/GSK3 and other similar signaling pathways.[15, 33, 37] However, the role of Akt/GSK3 in neurobehavioral and neurochemical changes induced by duloxetine has yet to be fully clarified. According to our findings, duloxetine and other similar agents might act through these pathways (Akt/GSK3) and rescue cell survival and trigger neuroprotection. These novel results give us new insights into the molecular effects of duloxetine in hippocampal cells.

Conclusion

In light of the results of the present study, we can conclude that the chronic administration of methamphetamine in adult rats causes disturbances in learning and spatial memory and can trigger the activation of oxidative stress and inflammation. According to our data, the inhibition of Akt, which causes the activation of GSK3, might be responsible for this type of neurodegeneration. In addition, our data indicated that duloxetine could act against methamphetamine-induced cognition impairment and neurodegeneration. It is worthy of note that, for the first time, the current study showed that the Akt/GSK3 signaling pathways might be involved in the protective effects of duloxetine against methamphetamine-induced neurobehavioral and biochemical malicious effects. Although these findings confer a new insight into the hitherto unknown mechanisms of methamphetamine-induced neurodegeneration, further evaluation of the precise molecular and

cellular aspects of the protective properties of duloxetine against methamphetamine-induced neurodegeneration and cognition impairment seems necessary.

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