

Effects of doxepin on brain-derived neurotrophic factor, tumor necrosis factor alpha, mitogen-activated protein kinase 14, and AKT1 genes expression in rat hippocampus

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Abstract

Background: It has been suggested that doxepin in addition to enhancement of noradrenaline and serotonin levels may have neuroprotective effects. Therefore, this study investigated the effect of doxepin on gene expression of brain-derived neurotrophic factor (BDNF), tumor necrosis factor alpha (TNF- α), mitogen-activated protein kinase 14 (MAPK14), and serine-threonine protein kinase AKT1 in rat hippocampus.

Materials and Methods: Male rats were divided randomly into three groups: Control, doxepin 1 mg/kg, and doxepin 5 mg/kg. Rats received an i.p injection of doxepin for 21 days. Then the hippocampi were dissected for the measurement of the expression of BDNF, TNF- α , MAPK14, and AKT1 genes.

Results: Our results showed no significant effects of doxepin on gene expression of BDNF, TNF- α , MAPK14, and AKT1 genes in the hippocampus.

Conclusions: These results did not show significant effects of doxepin on the genes that affect the neuronal survival in intact animals. However, more studies need to be done, especially in models associated with neuronal damage.

Key Words: AKT1, brain-derived neurotrophic factor, doxepin, hippocampus, mitogen-activated protein kinase 14, tumor necrosis factor alpha

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INTRODUCTION

Although antidepressants are an effective treatment for depression, their therapeutic mechanisms are not entirely understood. The initial hypothesis was the enhancement of some neurotransmitters levels such as noradrenaline and serotonin. Although this assumption is correct, but there is no explanation for 2–3 weeks delay phase between starting of treatment and the

appearance of therapeutic effects. Furthermore, the depletion of monoamines do not cause depression in healthy people.^[1] It seems that other mechanisms must also be involved. Many studies have demonstrated that cellular and molecular adaptations occur in the brain at different levels of response to treatment with antidepressants. It has been reported that antidepressants have neuroprotective effects.^[2,3] They

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reduce stress-induced atrophy of hippocampal CA3 pyramidal cells^[4,5] and increase proliferation of granular cells in the hippocampus.^[6] Furthermore, recently it has been specified that antidepressants such as fluoxetine, clomipramine, amitriptyline, and desipramine have anti-inflammatory effects.^[7-9] It is noteworthy, chronic inflammatory diseases increase depression^[10-12] and the changes of immune system activity play an important role in the pathogenesis of depression.^[13,14]

Doxepin is a tricyclic antidepressant that reduces the reuptake of norepinephrine and serotonin. It is used to treat depression and anxiety disorders.^[15] Up to now various properties of doxepin have been reported, such as anti-inflammatory^[16] and anticonvulsant effects.^[17] Furthermore, doxepin played a role as a protective agent against oxidative stress, and it increased antioxidants.^[18] Furthermore, it is possible that antidepressant, such as doxepin can change genetics function of the hippocampus.^[19]

Since in our previous study, we observed that doxepin by doses of 1 and 5 mg/kg could increase learning and memory;^[20] Hence, we decided to study the mechanism of improvement of learning and memory by evaluating the expression of the genes of neurotrophic and proinflammatory proteins, and their probable signaling in the hippocampus. It is worth mentioning that studies have shown that any factor increase adult neurogenesis or prevent neuronal apoptosis in the hippocampus could effective in improving learning and memory.^[21] Studies have shown that brain-derived neurotrophic factor (BDNF) plays a role in regulating adult hippocampal neurogenesis, and this factor has been reported, that is, increased in chronic treatment with a different types of antidepressants in the hippocampus.^[22] It has been shown that the mitogen-activated protein kinase (MAPK) pathway plays a role in the signaling pathway of BDNF function.^[23] In addition, the role of serine-threonine protein kinase (AKT) signaling in the performance of some antidepressants has been shown.^[24] Since the anti-inflammatory role of doxepin in nonnervous tissues is known, in this study, we investigated the gene expression of tumor necrosis factor alpha (TNF- α) in the hippocampus. Perhaps these factors can also affect the MAPK signaling pathway in inflammatory processes in the brain.^[25,26]

Most of the protective effects of doxepin have been investigated *in vitro* conditions, and protective effects of doxepin *in vivo* conditions such as gene expression in the central nervous system are still unclear. Therefore, this study investigated the effect of doxepin on gene expression of BDNF, TNF- α , MAPK14, and AKT1 in rat hippocampus.

MATERIALS AND METHODS

Subjects

Twenty-one male Wistar rats (250–350 g) were housed five per cage and maintained on a 12 h light–dark cycle in an air conditioned constant temperature (23°C \pm 1°C) room, with food and water made available *ad libitum*. The Ethic Committee for Animal Experiments at Isfahan University approved the study and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Animals were divided into three groups ($n = 7$ in each group): The control, the doxepin 1 mg/kg, and the doxepin 5 mg/kg. Doxepin was dissolved in saline. The rats received an intraperitoneal injection of doxepin (in the doxepin groups) or an equal volume of saline (in the control group) daily between 7 and 8 a.m for a period of 21 days.^[20]

Then rats were lightly anesthetized with diethyl ether inhalation and sacrificed by decapitation. Their brains were immediately removed from the skull, and both hippocampi were instantly dissected. The extracted hippocampus tissues removed on a cold artificial cerebrospinal fluid and got deep freeze in liquid nitrogen, and then stored at -80°C until further studies.

Gene expression assessment

Real-time polymerase chain reaction (PCR) was used to evaluate the expression of BDNF, TNF- α , MAPK14, and AKT1 genes in both hippocampi. Total RNA was isolated from hippocampus tissues using YTA kit (Yekta Tajhiz Azma, IRAN), according to the manufacturer's instructions. After isolation, the quality of messenger RNA (mRNA) was checked by gel electrophoresis, and RNA quantity was measured using nanodrop (OD 260 nm). At the reverse transcription step, 5 ng of total RNA was used to synthesis the complementary DNA with random hexamers primer using the Reverta-L kit (Amplisens, Moscow, Russia), according to the manufacturer's manual.

The real-time PCR was performed using the StepOnePlus real-time PCR System (Applied Biosystems). RealQ Plus 2x Master Mix Green with high ROX™ (Ampliqon) and specific primers were used [Table 1]. Beta-actin (ACTB) was used as an internal control to normalize RNA input. Cycle parameters for real-time PCR included 95°C for 1 min, 95°C for 15 s and 60°C for 60s. The C_t value is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. The fold change was calculated using the $2^{-\Delta\Delta C_t}$ method

Table 1: Primers used in real-time PCR experiments

Primers	Sequence (5'-3')
ACTB	Forward AGGCCCTCTGAACCTAAG
	Reverse CCAGAGGCATACAGGGACAA
TNF α	Forward ACGTCGTAGCAAACCACCAA
	Reverse CAAGGGCTCTTGATGGCAGA
BDNF	Forward AGAATGAGGGCGTTTGCCTA
	Reverse CCTGGTGAACATTGTGGCT
MAPK 14	Forward CCGAGCGATACCAGAACCT
	Reverse CTTCAGTCCACACGATGTC
AKT1	Forward TCGTGTGGCAAGATGTGTATGAGA
	Reverse CAGGCGGCGTGATGGTGAT

PCR: Polymerase chain reaction, ACTB: Beta-actin, TNF α : Tumor necrosis factor alpha, BDNF: Brain-derived neurotrophic factor, MAPK14: Mitogen-activated protein kinase 14, AKT1: (V-akt murine thymoma viral oncogene homolog 1) gene that encode RAC-alpha serine/threonine-protein kinase

presented as the fold expression change in treated experiment group relative to their corresponding control group after normalization to the ACTB endogenous control.

Data analysis

Data were analyzed using both the SPSS 21 (IBM Corporation) for Windows and the Rest 2009 (developed by M. Pfaffl (Technical University Munich) and QIAGEN). The results analyzed for gene expression with one sample *t*-test between the treated and the control groups and with unpaired *t*-test between the two treated groups. The significant level was set at $P < 0.05$. Results are expressed as mean \pm standard error of the mean.

RESULTS

Gene expression of brain-derived neurotrophic factor, tumor necrosis factor alpha, mitogen-activated protein kinase 14, and AKT1 after doxepin

The chronic injection of doxepin 1 mg/kg showed no significant enhancements of BDNF (1.42 fold), TNF- α (1.66 fold), MAPK14 (1.31 fold), and AKT1 (1.16 fold) mRNA levels compared to the control group [Figures 1-4].

As seen in Figures 1-4, the BDNF and TNF- α mRNA expressions (7.83 and 2.59 fold, respectively) no significantly increased compared to the control group by the chronic injection of doxepin 5 mg/kg, whereas, the MAPK14 and AKT1 mRNA expressions (0.57 and 0.81 fold, respectively) showed no significant decrement in compared with the control group [Figures 1-4].

DISCUSSION

Results showed that doxepin had no detectable effects on the expression of BDNF, TNF- α , MAPK14, and AKT1 genes [Figures 1-4]. Therefore, doxepin

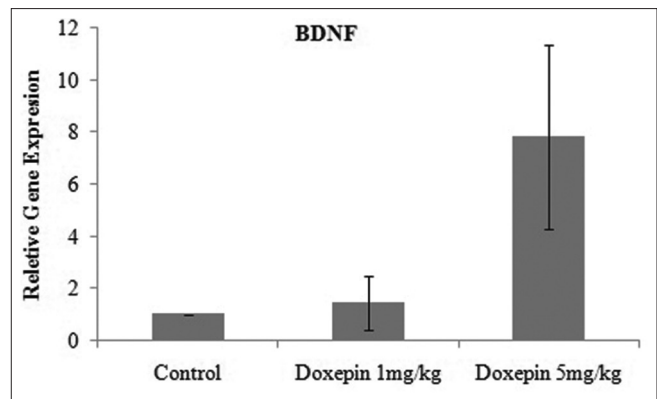


Figure 1: Effects of doxepin on relative gene expression of brain-derived neurotrophic factor in rat hippocampus ($n = 7$)

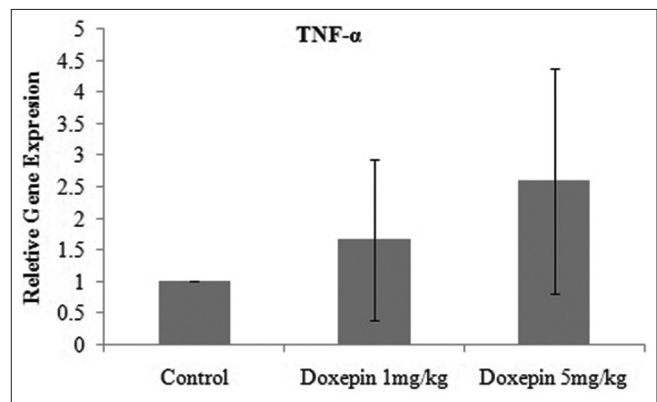


Figure 2: Effects of doxepin on relative gene expression of tumor necrosis factor alpha in rat hippocampus ($n = 7$)

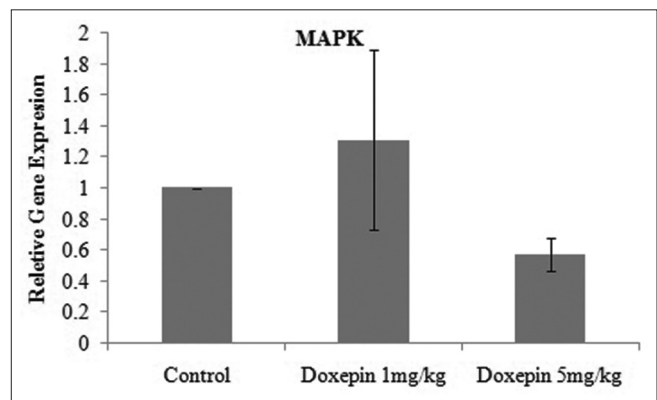


Figure 3: Effects of doxepin on relative gene expression of mitogen-activated protein kinase 14 in rat hippocampus ($n = 7$)

may not be able to affect the survival of neurons in the hippocampus of intact rats. The previous studies have shown that doxepin significantly inhibited the cell death in the cultured neurons, against oxidative stress-induced injury.^[18] It seems that doxepin has neuroprotective effects, as it has been demonstrated in a model of focal cerebral ischemia-reperfusion injured rats.^[27] In the present study, there were any significant changes on the expression of BDNF, TNF- α , MAPK14,

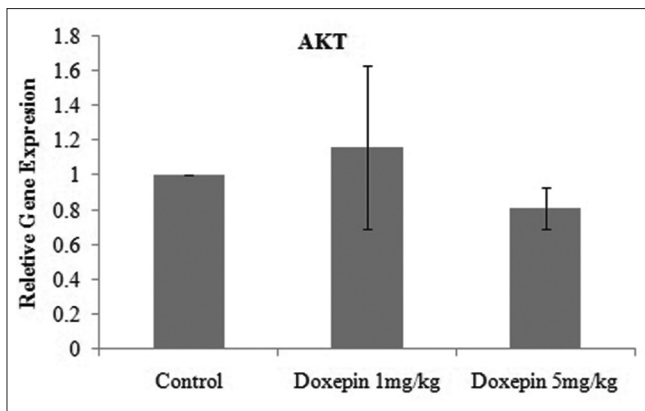


Figure 4: Effects of doxepin on relative gene expression of serine-threonine protein kinase AKT1 in rat hippocampus ($n = 7$)

and AKT1 genes, whereas, in most previous studies, the protective effects of antidepressants were observed in depression. As one reason for these different responses, it was coincided a background of cell death or a nerve damage.^[2,3,28,29] Hence, it is possible that if there is a degenerative disorder, doxepin can also provide protective effects.

It is revealed that treatment with some antidepressants changed the levels of some genes that are involved in the survival of neurons.^[30] However, the effects of antidepressants on different genes are complex and presumably depend on the antidepressant type.^[31] It has been reported that a different form of antidepressant is able to prevent or reverse apoptotic effects. Some studies demonstrated that antidepressants had a strong effect on adult neurogenesis.^[19]

The BDNF is active in the hippocampus and induces the survival, development, and the function of neurons and neuronal synapses.^[32] It has been suggested that antidepressants affect the expression of BDNF in hippocampal neurons,^[33] and neurotrophic effects of antidepressants are directly or indirectly mediated by BDNF.^[29,34] The previous studies found that repeated injections of serotonin transporter inhibitors (fluoxetine, paroxetine, and sertraline) and monoamine oxidase inhibitors have a dual effect on the expression of the BDNF gene in rat hippocampus during short- and long-time.^[35] Furthermore, there was no change in the expression of the BDNF gene following treatment with noradrenaline reuptake inhibitors (desipramine and maprotiline).^[35] Hence, it seems that the effects of antidepressants on BDNF gene expression are depending on the type of drug and usage time.

In this study, gene expression of TNF- α had no changes in the hippocampus, whereas, other reports demonstrated the anti-inflammatory effects of doxepin

in peripheral tissues.^[16,18] Since in this study, doxepin was used in healthy animals that had no background of inflammation, therefore, the TNF- α level did not change in all groups. However, these results do not rule out this possibility that doxepin leads to a reduction in the inflammatory process in the nervous tissue if there is a disease with inflammatory effects on the nervous system. MAPK14, also called p38- α , is implicated in a wide variety of cellular function, such as proliferation, differentiation, transcription regulation, and development.^[36]

It has been shown that antidepressants can differently modulate MAPK signaling pathway and maybe are the early targets of their action.^[37-39] AKT1 is involved in cellular survival pathways by inhibiting apoptotic processes, and its probable role in the actions of antidepressants.^[23]

CONCLUSION

The current results did not show significant effects of doxepin on the neuronal survival factors in intact animals. However, according to the previous results the neuroprotective effects of antidepressants in models of neuronal damage were shown. It seems that protective effects of doxepin in the presence of nerve damage cannot be ruled out. Therefore, more studies need to be done.

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Conflicts of interest

There are no conflicts of interest.

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