Original Article

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 ± 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 $2^{-\Delta\Delta Ct}$ method

Table 1: Primers used in real-time PCR experiments

Primers	Sequence (5'-3')
ACTB	Forward AGGCCCCTCTGAACCCTAAG
	Reverse CCAGAGGCATACAGGGACAA
TNFα	Forward ACGTCGTAGCAAACCACCAA
	Reverse CAAGGGCTCTTGATGGCAGA
BDNF	Forward AGAATGAGGGCGTTTGCGTA
	Reverse CCTGGTGGAACATTGTGGCT
MAPK14	Forward CCGAGCGATACCAGAACCT
	Reverse CTTCACTGCCACACGATGTC
AKT1	Forward TCGTGTGGCAAGATGTGTATGAGA
	Reverse CAGGCGGCGTGATGGTGAT

PCR: Polymerase chain reaction, ACTB: Beta-actin, TNFα: 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 ± 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

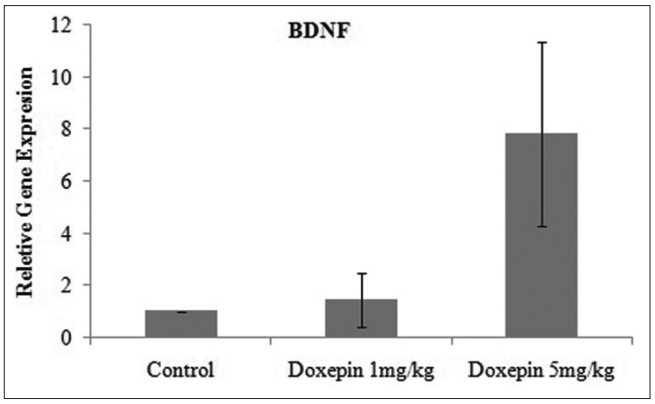

Figure 1: Effects of doxepin on relative gene expression of brain-derived neurotrophic factor in rat hippocampus ($n = 7$)

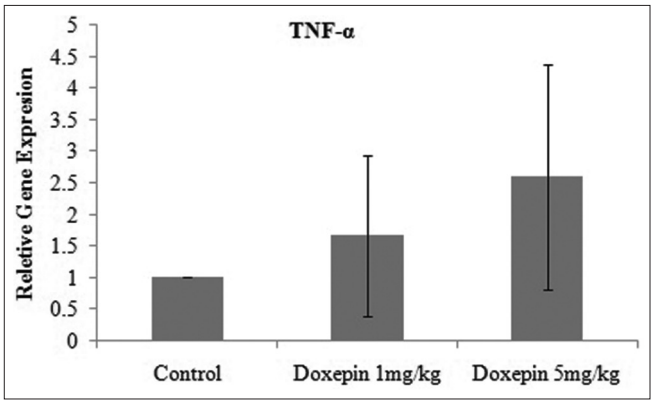

Figure 2: Effects of doxepin on relative gene expression of tumor necrosis factor alpha in rat hippocampus ($n = 7$)

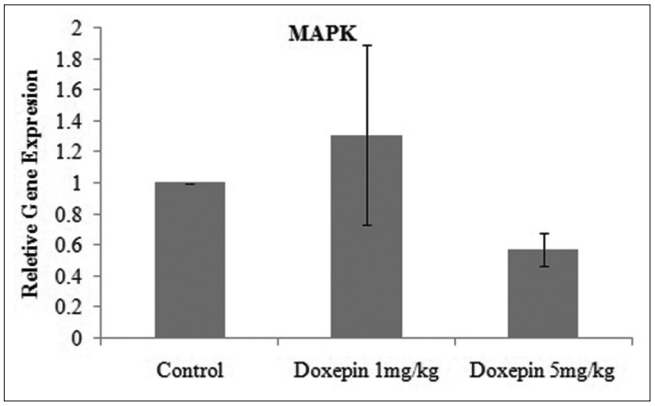

Figure 3: Effects of doxepin on relative gene expression of mitogen-activated protein kinase 14 in rat hippocampus ($n = 7$)

may not 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,

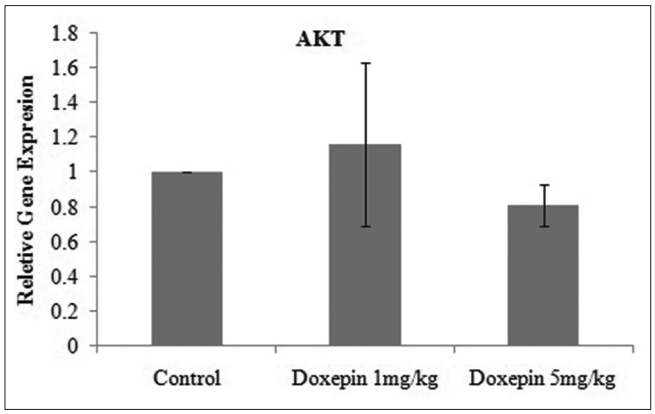

Figure 4: Effects of doxepin on relative gene expression of serine-threonine protein kinase AKT1 in rat hippocampus (n = 7)

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 (dsypramyn 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.

REFERENCES

1. Roumestan C, Michel A, Bichon F, Portet K, Detoc M, Henriquet C, *et al.* Anti-inflammatory properties of desipramine and fluoxetine. Respir Res 2007;8:35.
2. Jacobson MD, Raff MC. Programmed cell death and Bcl-2 protection in very low oxygen. Nature 1995;374:814-6.
3. Jin Y, Lim CM, Kim SW, Park JY, Seo JS, Han PL, *et al.* Fluoxetine attenuates kainic acid-induced neuronal cell death in the mouse hippocampus. Brain Res 2009;1281:108-16.
4. Duman RS, Malberg J, Thome J. Neural plasticity to stress and antidepressant treatment. Biol Psychiatry 1999;46:1181-91.
5. Xu H, Steven Richardson J, Li XM. Dose-related effects of chronic antidepressants on neuroprotective proteins BDNF, Bcl-2 and Cu/Zn-SOD in rat hippocampus. Neuropsychopharmacology 2003;28:53-62.
6. Watanabe Y, Gould E, Daniels DC, Cameron H, McEwen BS. Tianeptine attenuates stress-induced morphological changes in the hippocampus. Eur J Pharmacol 1992;222:157-62.
7. Bianchi M, Sacerdote P, Panerai AE. Chlomipramine differently affects inflammatory edema and pain in the rat. Pharmacol Biochem Behav 1994;48:1037-40.
8. Abdel-Salam OM, Baiuomy AR, Arbid MS. Studies on the anti-inflammatory effect of fluoxetine in the rat. Pharmacol Res 2004;49:119-31.

9. Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:201-17.
10. Manderbacka K, Sund R, Koski S, Keskimäki I, Elovainio M. Diabetes and depression? Secular trends in the use of antidepressants among persons with diabetes in Finland in 1997-2007. Pharmacoepidemiol Drug Saf 2011;20:338-43.
11. Zautra AJ, Yocum DC, Villanueva I, Smith B, Davis MC, Attrep J, *et al.* Immune activation and depression in women with rheumatoid arthritis. J Rheumatol 2004;31:457-63.
12. Ali S, Stone MA, Peters JL, Davies MJ, Khunti K. The prevalence of co-morbid depression in adults with Type 2 diabetes: A systematic review and meta-analysis. Diabet Med 2006;23:1165-73.
13. Castanon N, Leonard BE, Neveu PJ, Yirmiya R. Effects of antidepressants on cytokine production and actions. Brain Behav Immun 2002;16:569-74.
14. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: Inflammation and the pathogenesis of depression. Trends Immunol 2006;27:24-31.
15. Hajak G, Rodenbeck A, Voderholzer U, Riemann D, Cohrs S, Hohagen F, *et al.* Doxepin in the treatment of primary insomnia: A placebo-controlled, double-blind, polysomnographic study. J Clin Psychiatry 2001;62:453-63.
16. Drake LA, Fallon JD, Sober A. Relief of pruritus in patients with atopic dermatitis after treatment with topical doxepin cream. The Doxepin Study Group. J Am Acad Dermatol 1994;31:613-6.
17. Sun XY, Zhang L, Wei CX, Piao HR, Quan ZS. Characterization of the anticonvulsant activity of doxepin in various experimental seizure models in mice. Pharmacol Rep 2009;61:245-51.
18. Ji BS, Ji H, Liu GQ. Doxepin protects cultured neurons against oxidative stress-induced injury. Acta Pharmacol Sin 2004;25:297-300.
19. Lee MM, Reif A, Schmitt AG. Major depression: A role for hippocampal neurogenesis? Curr Top Behav Neurosci. 2013;14:153-79.
20. Gharzi M, Dolatabadi HR, Reisi P, Javanmard SH. Dose related effects of doxepin on passive avoidance learning in rats. Res Pharm Sci 2012;7:S35.
21. Arabpoor Z, Hamidi G, Rashidi B, Shabrang M, Alaei H, Sharifi MR, *et al.* Erythropoietin improves neuronal proliferation in dentate gyrus of hippocampal formation in an animal model of Alzheimer's disease. Adv Biomed Res 2012;1:50.
22. Balu DT, Hoshaw BA, Malberg JE, Rosenzweig-Lipson S, Schechter LE, Lucki I. regulation of central BDNF protein levels by antidepressant and non-antidepressant drug treatments. Brain Res 2008;1211:37-43.
23. Wang R, Li YH, Xu Y, Li YB, Wu HL, Guo H, *et al.* Curcumin produces neuroprotective effects via activating brain-derived neurotrophic factor/TrkB-dependent MAPK and PI-3K cascades in rodent cortical neurons. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:147-53.
24. Beaulieu JM, Gainetdinov RR, Caron MG. Akt/GSK3 signaling in the action of psychotropic drugs. Annu Rev Pharmacol Toxicol 2009;49:327-47.
25. Li YP, Chen Y, John J, Moylan J, Jin B, Mann DL, *et al.* TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. FASEB J 2005;19:362-70.
26. Chaparro-Huerta V, Rivera-Cervantes MC, Flores-Soto ME, Gómez-Pinedo U, Beas-Zárate C. Proinflammatory cytokines and apoptosis following glutamate-induced excitotoxicity mediated by p38 MAPK in the hippocampus of neonatal rats. J Neuroimmunol 2005;165:53-62.
27. Ji BS, Ji H, Liu GQ. Protective effects of doxepin on focal cerebral ischemia-reperfusion injured rats. Chin Pharmacol Bull 2003;19:896-8.
28. Merry DE, Korsmeyer SJ. Bcl-2 gene family in the nervous system. Annu Rev Neurosci 1997;20:245-67.
29. Drzyzga LR, Marcinowska A, Obuchowicz E. Antiapoptotic and neurotrophic effects of antidepressants: A review of clinical and experimental studies. Brain Res Bull 2009;79:248-57.
30. McKernan DP, Dinan TG, Cryan JF. "Killing the Blues": A role for cellular suicide (apoptosis) in depression and the antidepressant response? Prog Neurobiol 2009;88:246-63.
31. Djordjevic A, Djordjevic J, Elakovic I, Adzic M, Matic G, Radojcic MB. Effects of fluoxetine on plasticity and apoptosis evoked by chronic stress in rat prefrontal cortex. Eur J Pharmacol 2012;693:37-44.
32. Yamada K, Nabeshima T. Brain-derived neurotrophic factor/TrkB signaling in memory processes. J Pharmacol Sci 2003;91:267-70.
33. Peng CH, Chiou SH, Chen SJ, Chou YC, Ku HH, Cheng CK, *et al.* Neuroprotection by Imipramine against lipopolysaccharide-induced apoptosis in hippocampus-derived neural stem cells mediated by activation of BDNF and the MAPK pathway. Eur Neuropsychopharmacol 2008;18:128-40.
34. Zhang Y, Gu F, Chen J, Dong W. Chronic antidepressant administration alleviates frontal and hippocampal BDNF deficits in CUMS rat. Brain Res 2010;1366:141-8.
35. Coppell AL, Pei Q, Zetterström TS. Bi-phasic change in BDNF gene expression following antidepressant drug treatment. Neuropharmacology 2003;44:903-10.
36. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, *et al.* Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. Endocr Rev 2001;22:153-83.
37. Di Benedetto B, Radecke J, Schmidt MV, Rupprecht R. Acute antidepressant treatment differently modulates ERK/MAPK activation in neurons and astrocytes of the adult mouse prefrontal cortex. Neuroscience 2013;232:161-8.
38. Chou CT, He S, Jan CR. Paroxetine-induced apoptosis in human osteosarcoma cells: Activation of p38 MAP kinase and caspase-3 pathways without involvement of [Ca2+]i elevation. Toxicol Appl Pharmacol 2007;218:265-73.
39. Kodama M, Russell DS, Duman RS. Electroconvulsive seizures increase the expression of MAP kinase phosphatases in limbic regions of rat brain. Neuropsychopharmacology 2005;30:360-71.