Original Article

Effects of different doses of doxepin on passive avoidance learning in rats

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Abstract

Background: Studies have shown that Doxepin has anti-inflammatory effects and reduces oxidative stress. Due to the fact that other tricyclic antidepressants have been shown to have neuroprotective effects, this study aimed to investigate the effects of different doses of doxepin on passive avoidance learning in rats.

Materials and Methods: Old male Wistar rats were used in this study. Doxepin was administered intraperitoneally (1, 5 and 10 mg/kg) for 21 days. Passive avoidance learning test was used for evaluation of learning and memory. Rats received foot electrical shock on fifteen day, and step through latencies were evaluated one week after the electrical shock in retention phase.

Results: Administration of Doxepin considerably increased the step through latencies in the rats that received the doses of 1 and 5 mg/kg ($P < 0.05$). However, in the dose of 10 mg/kg, there wasn't any significant change comparing to control group.

Conclusion: These results indicate that Doxepin has desirable effects on cognitive functions in low doses. Therefore, Doxepin can be considered as memory enhancers that understanding the underling mechanisms need further investigation.

Key Words: Doxepin, learning and memory, rat, tricyclic antidepressant

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INTRODUCTION

Although antidepressants are used to treat depression, their therapeutic mechanism is not fully understood. The primary hypothesis for the action of antidepressants was enhancement of noradrenaline and serotonin levels. Although this seems to be true, but there is no explanation for the 2 to 3 weeks delayed phase of treatment; moreover monoamine depletion in healthy individuals does not cause depression,[1] therefore, other mechanisms must be involved. Many studies have shown that neurons make adaptations in response to treatment with antidepressants in the brain at different levels of cellular and molecular. It has been reported that antidepressants reduce atrophy of hippocampal CA3 pyramidal cells induced by stress[2,3] and increase hippocampal granular cells neurogenesis.[4] Also, it has been recently shown that antidepressant drugs like fluoxetine, clomipramine, amitriptyline and desipramine have anti-inflammatory effects, and many studies have demonstrated these anti-inflammatory effects.[5-7] It has been demonstrated chronic inflammatory

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diseases increase risk of depression[8-10] and changes in immune system activity play a significant role in the pathogenesis of depression.[11,12]

Studies have shown that fluoxetine, a selective serotonin reuptake inhibitor (SSRI) antidepressant, specifically prevents cerebral delayed injury after ischemia.[13] In animal model of kainic acid-induced cell death fluoxetine has significantly prevented neuronal death and has strongly suppressed gliosis (growth of astrocytes in damaged areas of the central nervous system) and proinflammatory markers.[14] It has been shown that as dose-dependent, amitriptyline and venlafaxine that are serotonin-norepinephrine reuptake inhibitor antidepressants, increase brain-derived neurotrophic factor (BDNF) expression and Bcl-2 (B-cell lymphoma 2) and these factors promote neuronal regeneration in the mammalian central nervous system and have neuroprotective effects;[15-17] also they increase copper-zinc superoxide dismutase that has neuroprotective effects in hippocampus in brain damages.[1] Thus they have favorable effects on survival and function of neurons in the hippocampus.

In addition, maprotiline, a potent inhibitor of norepinephrine reuptake antidepressant, has recently been shown to prevent inflammation significantly if used as systemic or centrally (intracerebroventricular).[18] In this regard, studies have shown that maprotiline can prevent neuronal death in Huntington's disease through the favorable effect on mitochondria and reduction of its permeability during apoptosis.[19]

Doxepin that is a member of tricyclic antidepressants family is a norepinephrine and serotonin reuptake inhibitor. Doxepin is used to treat depression and anxiety disorders and low doses of it are used to treat sleep disorders. Doxepin also has been known as second-line therapy for chronic urticaria.[20] Various properties of doxepin have been reported; among them we can mention the anticonvulsant effects. The patients who have been treated with doxepin for more than three years show an improvement in control of seizure.[21] In addition, it also has anti-inflammatory effects and topical application of doxepin has therapeutic effects on atopic dermatitis.[22] Doxepin as a protective agent against oxidative stress is also discussed. It has been observed in an *in vitro* study that doxepin in the medium protects neurons against oxidative stress; Doxepin make protection against oxidative stress through reduction of calcium signaling that is a key factor in the damages caused by oxidative stress.[23,24] In addition, doxepin increases antioxidants such as superoxide dismutase (SOD) and reduces lipid peroxide.[24]

Oxidative stress that mediated by oxygen reactive species (ROS) is due to imbalance between ROS production and activity of protective mechanisms. Oxidative stress has been identified that plays a clear role in the aging process[25] as well as some central nervous system diseases, such as Parkinson's disease[26] and Alzheimer's.[27] Therefore, according to various hypotheses on the effects of antidepressants in reducing oxidative stress and inflammatory factors induced by this process, the aim of this study was to investigate the effect of doxepin on avoidance learning and memory in old rats.

MATERIALS AND METHODS

Male Wistar rats 350-400g (1 year old, rats were purchased from the Isfahan Laboratory Animal stock) were housed four per cage and maintained on a 12 h light–dark cycle in an air conditioned constant temperature (23 ± 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 four groups ($n = 8$ in each group): The control, doxepin 1 mg/kg (Ray Chemicals Pvt. Ltd.), doxepin 5 mg/kg and doxepin 10 mg/kg. Doxepin was dissolved in saline and was injected intraperitoneally. Rats received doxepin for 21 days. Animals in the control group received same volume of placebo. Then the rats were evaluated using passive avoidance learning test.

The apparatus consists of two separate chambers connected through a guillotine door. One chamber was illuminated, while the other was dark. The floor of both the chambers consists of steel grids, used to deliver electric shocks. On the acquisition trial, each rat was placed in illuminated chamber while its back was to the guillotine door. After 10 s of habituation, the guillotine door separating the illuminated and dark chambers was opened and the initial latency to enter the dark chamber was recorded. The guillotine door was closed immediately after the rat enters the dark chamber, and an electric foot shock (75 V, 0.2 mA, 50 Hz) was delivered to the floor grids for 3 s, then the rat was removed from the dark chamber and returned to its home cage. Twenty four hours later, retention latency time to enter the dark chamber was taken in the same way as in the acquisition trial, but foot shock was not delivered, and the latency time was recorded up to a maximum of 600 s.[28]

The data were analyzed statistically by *t*-test and one way analysis of variance (ANOVA) with Bonferroni's

post hoc statistical tests. The significant level was set at $P < 0.05$. Results are expressed as mean ± S.E.M.

RESULTS

The mean initial latency in the acquisition trial wasn't different among the groups significantly [control: 25.4 ± 11.96; Doxepin 1, 5, 10 mg: 29.4 ± 5.08, 17.8 ± 5.91, 18.78 ± 5.91 respectively; $P = 0.43$; Figure 1]. Results from the retention phase of PAL as measured by mean retention latency time have shown twenty four hours after acquisition phase, mean retention latencies except in the Doxepin treated group 10 mg/kg (352.9 ± 89.98 s) was increased in Doxepin treated groups 1 mg/kg (549.12 ± 30.53 s; $P < 0.05$) and 5 mg/kg (553.57 ± 31.83 s; $P < 0.05$) than the control group (398.5 ± 72.3 s). Also, mean retention latency time was further significantly ($P < 0.05$) in Doxepin treated groups 1 mg/kg and 5 mg/kg comparing to the Doxepin treated group 10 mg/kg [Figure 2].

DISCUSSION

The results suggest that, although high dose of doxepin has no positive effect on memory, but it significantly improves memory in low doses. These results can be interpreted with regard to the effects of antidepressants on brain processes. Rats were tested in this study were aged and we know that aging is associated with gradual neuronal death and reduction of the brain capabilities, including memory. The effect of doxepin to improve memory in rats probably is due to its effects on the processes associated with aging. Studies have been proposed several hypotheses for aging. One of these assumptions is the free radical theory. This theory states that aging of the organisms is due to accumulation of damages associated to free radical in the cells over time.[29] Free radicals cause inflammation and inflammation process induces production of free radicals and reduces antioxidant capacity of cells. Excessive produced free radicals interact with cell membrane fatty acids and proteins and make disrupt their performance. In addition, these radicals can cause DNA damage that can cause cancer and age-related disorders.[30] Recent studies suggest that aging is associated with chronic low-grade inflammation. This inflammation caused by the imbalance between inflammatory and anti-inflammatory mediators and production of cell-mediated inflammation.[31] So it seems that anti-inflammatory drugs may delay the process of aging and its side effects, such as loss of memory. Inflammation is part of the non-specific response of the body's response to any type of injury that is self-limiting in normal conditions but in some disorders it continues to be chronic.[32] On the other hand, researches have shown inflammation is associated with many diseases, including Alzheimer's disease, multiple sclerosis and Parkinson's disease.[33] It has been suggested that chronic systemic inflammation accelerates the onset and progression of neurodegeneration. A possible explanation for this event is that systemic inflammation induces changes in phenotype of microglias from the relatively benign to the tissue-destructive phenotype.[34]

Studies have shown a link between inflammation and depression. For example, in a meta-analysis on 50 studies, most studies have shown that depressed patients have an increase in inflammatory cytokines, IL-6 and acute phase proteins.[35] Studies on Antidepressants have been shown that some of these drugs also have anti-inflammatory properties. For example, it was shown that paroxetine, an antidepressant drug that is selective serotonin reuptake inhibitors, may have anti-inflammatory effects and inhibits oxidative stress-mediated glial activity. This has led to offer paroxetine and its analogues as drugs to treat Parkinson's disease that is associated with neuronal inflammation.[36] Also, studies have shown that doxepin, a tricyclic

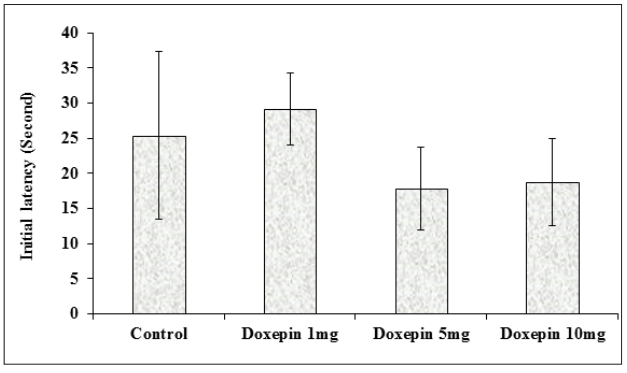

Figure 1: Dose-related effects of doxepin on initial latency in rats. Data are expressed as mean ± SEM. ($n = 8$)

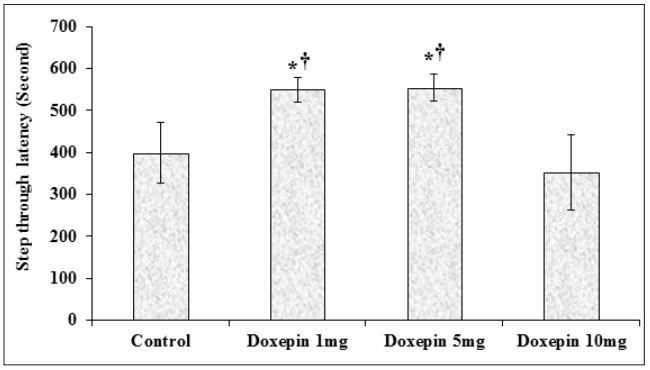

Figure 2: Dose-related effects of Doxepin on step-through latency in rats 24 h after PA acquisition. Data are expressed as mean ± SEM. ($n = 8$). *$P < 0.05$ with respect to the control group, †$P < 0.05$ with respect to the Doxepin 10 mg group

antidepressant, has anti-inflammatory effects. Topical application of doxepin alone or in combination with triamcinolone acetonide is effective for the treatment of atopic dermatitis.[22] Also antioxidative stress of doxepin has been demonstrated *in vitro* that indicating the effectiveness of this substance on neurodegenerative disorders.[24] With regards to the anti-inflammatory effects doxepin, it seems that this drug may improve the function of nervous system and memory by reducing the inflammation.

It is well known that doxepin has positive effects on sleep and studies have shown its beneficial effects on patients with insomnia.[37,38] Recent studies have demonstrated the involvement of sleep in memory formation. It seems that hippocampal activities through specific coordinated neuropsychological processes facilitates entry of new information into pre-existing network of cortex and thereby contribute to memory consolidation during sleep.[39] When human is awake during appropriate biological times, human emotional and cognitive performance can be improved. On the other hand, when awakening occurs at the interface between inappropriate biological times (such as disorders of circadian sleep rhythm), it induces cognitive, emotional and learning disorders.[40] Saletin and colleagues showed that although sleep doesn't generally consolidate all the information, but it purposefully improves some parts of memory, while actively causes amnesia of other parts.[41]

Other studies have shown that tricyclic antidepressants through a variety of mechanisms improved diseases that affect memory. For example, studies have shown that imipramine through prevention of beta-amyloidal and TNF-alpha accumulation prevents memory deficits and could be a candidate for the treatment of Alzheimer's.[42] Also, another study on another tricyclic antidepressant, amitriptyline, which performed in aging and cognitive impairment in Alzheimer's rats, showed its positive effects in the brains and it improved cognitive functioning as well as short-term and long-term memory.[43]

The results of this study showed that high doses of doxepin has no significant effect on memory and this could be due to toxic effects of high doses of the drug. Many studies using different approaches show differences in relative toxicity of antidepressants. There are evidences. that although tricyclic antidepressants are effective in treating various clinical disorders, in comparison with other antidepressants have side effects even at doses prescribed[44] and in this regard, it have been identified that doxepin is more toxic than amitriptyline.[45] Studies suggested that doxepin and other tricyclic antidepressants inhibit the enzyme Glutathione S-transferase pi in parietal and frontal cortex, hippocampus and brain stem. It should be noted that the normal enzyme Glutathione S-transferase pi acts as a barrier and prevents the brain from being exposed to the electrophiles. Therefore, the inhibition of this enzyme by tricyclic antidepressants (especially at high doses), caused brain damage by exposure to electrophilic reactions.[46] However, further studies should be done to evaluate the potential toxicity.

In general, present study suggests doxepin as a drug with potential positive effects on memory; however, further studies are needed to clarify the mechanism of action. Studies have been conducted on the effects of doxepin on oxidative stress was limited to *in vitro*, therefore evaluation of its effects as *in vivo* can confirm these conclusions with more confidence.

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