Original Article ICNSJ

Effect of Magnetic Tacrine-Loaded Chitosan Nanoparticles on Spatial Learning, Memory, Amyloid Precursor Protein and Seladin-1 Expression in the Hippocampus of Streptozotocin-Exposed Rats

Golamreza Hassanzadeh[1], Zahra Fallahi[2], Mohammad Khanmohammadi[3], Hamideh Elmizadeh[3], Mohammad Sharifzadeh[4], Kosar Nouri[2], Zahra Heydarian[2], Simin Mahakizadeh[1], Adib Zendedel[5], Cordian Beyer[5], Homa Mohseni Kouchesfahani[2,6]

[1] Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran
[2] Department of Animal Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
[3] Department of Analytical Chemistry, Faculty of Chemistry, Imam Khomeini
[4] Department of Pharmacology & Toxicology, Faculty of Pharmacy, Tehran University of medical sciences, Tehran, Iran
[5] Institute of Neuroanatomy, Faculty of Medicine, RWTH Aachen University, Germany
[6] Department of Animal Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory and cognitive dysfunction due to neuronal cell loss in higher brain centers. Senile plaques containing amyloid β (Aβ) are associated with this disease as well as a reduction in cholinergic neuron numbers. Tacrine is a reversible cholinesterase inhibitor in clinical use to treat moderate forms of AD. Chitosan nanoparticles represent an effective systemic delivery system for drugs. The application of tacrine-loaded chitosan nanoparticles has been shown to selectively increase tacrine concentrations in the brain tissue. In this study, we compared magnetic and non-magnetic tacrine-loaded chitosan nanoparticles for their bioactivity and neuroprotective potency in streptozotocin (stz)-induced neurodegeneration, an accepted animal model for AD. Male rats received a single injection of stz via an implanted cannula into the lateral brain ventricle. Tacrine (tac)-loaded chitosan nanoparticles were delivered into the tail vein. Spatial learning and memory were analyzed using the Morris water maze task. Amyloid precursor protein gene (APP) and seladin-1 gene expression were studied in the hippocampus by real time-PCR. Tac-loaded non-magnetic and tac-loaded magnetic chitosan nanoparticles improved spatial learning and memory after stz treatment with magnetic nanoparticles being most effective. Similarly, tac-loaded chitosan nanoparticles increased seladin-1 and reduced APP gene expression. Again, magnetic nanoparticles were more effective. These data reveal that tac-loaded non magnetic and tac-loaded magnetic chitosan nanoparticles to a higher extent improve brain deficits related to stz application. We conclude that the magnetic target drug delivery system is a promising therapeutic strategy to protect AD-related degenerating in the CNS.

Keywords: Alzheimer; Nanoparticles; Tacrine; Chitosan; Morris Water Maze; Seladin-1; Amyloid-ß; Hippocampus

ICNSJ 2016; 3 (1):25-31 www.journals.sbmu.ac.ir/neuroscience

Correspondence to: Homa Mohseni Kouchesfahani, Department of Animal Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran; E-mail: kouchesfehani@yahoo.com
Received: Apr 2016 **Accepted:** May 2016

INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by a massive loss of synapses and dysfunction of the cholinergic neurotransmitter

system in the central nervous system (CNS) [1]. In addition, glutamatergic and serotonergic neurons are also affected [2]. Thus, cell-cell communication within the brain is first impaired and finally intrinsic neural networks become destroyed [3]. This is paralleled by a progressive decline of such as memory, learning and emotionality, and finally ends in dementia [3,4]. AD represents a worldwide major challenge for health care systems. At the histopathological level, depositions of amyloid-ß (Aβ) protein and the formation of neurofibrillary tangles (NFT) containing hyper-phosphorylated Tau protein are important hallmarks of AD (5-7). Besides other mechanisms which are currently under intensive scientific debate, these pathological processes are thought to play a major role in induction of neuronal cell death in the cerebral cortical hemispheres which lead to a dramatic shrinkage of the brain mass [8,9].

Much effort has been put into AD research to uncover the biochemical and molecular processes causing neuronal damage and death [10]. Despite an ongoing controversial debate on the role of Aß deposition and NFT for AD, most therapeutic strategies are designed to target these abnormal structures and to prevent Aß formation or to remove deposits [11-13]. However, AD appears as a multifactorial syndrome with several target proteins contributing to its etiology. Two main classes of medication appear to be suited for pharmacological management of AD. Agents which affect glutamatergic neurotransmission and therapeutic inhibitors of acetylcholinesterase (AChE) [1,14]. These drugs improve symptoms but only show a partial and limited long-lasting disease-modifying effect [15]. In recent years, several approaches like introduction of AChE inhibitors, such as tacrine, donepezil, galantamine, and rivastigmine as well as stimulating the immune system to remove Aß from the brain aimed at inhibiting AD disease progression [16]. These studies have been performed in experimental animal models and clinical trials [17]. The cholinergic hypothesis claims that the observed decrease of acetylcholine (ACh) in the brain of AD patients plays an important role in the deterioration of cognitive functioning [18]. Tacrine (tac) is a well-known and potent non-selective cholinesterase inhibitor and widely marketed to counteract and compensate for the loss of memory and intellectual decline in AD by improving ACh neurotransmission [17].

An important issue in systemic therapeutic pharmacological treatment of brain diseases is the reduction and/or diminishment of peripheral side effects and the precise local accumulation of the therapeutic agent in the relevant CNS structures. In the past years, polymeric-controlled drug delivery systems have evolved [10]. Although there are several advantages offered by controlled drug release, a major problem associated with the currently available systems are the obtained release rates which are either constant or decrease with time. Instead, a rapid and preferential target-intrinsic or in the vicinity of the focused area accumulation with the option of delivery on demand would be beneficial for a therapeutically optimum performance. This can be at best achieved by using delivery systems which can be externally controlled by magnetic tools [19]. Magnetically-targeted drug delivery system is a promising way which involves binding the drug to a small biocompatible magnetically-active component, and the application of a high-gradient magnetic field. This approach allows to accumulate the therapeutic agent in the target region by increasing the microvascular permeability and interstitial penetration [19,20].

The aim of the present study was to demonstrate (i) the improved bioactivity and neuroprotective potential of tacrine-loaded chitosan nanoparticles in an animal model consisting of streptozotocin (stz)-induced neurodegeneration in the cerebral cortex and (ii) the higher efficacy of magnetic vs. non-magnetic application forms of tac-loaded nanoparticles. Morris water maze testing was performed for functional behavioral outcome and expression levels of amyloid precursor protein (APP) and seladin-1 were measured by quantitative PCR.

MATERIAL AND METHODS

Animals

Ten week-old male Wistar rats (200–250 g) were maintained in a pathogen-free environment with free access to water and food following approval by the institutional animal experimentation ethical committee. Animals underwent routine cage maintenance once a week and microbiological monitoring according to the Islamic Republic of Iran Laboratory Animal Science Association recommendations. Research and animal care procedures were approved by the Review Board for the Care of Animal Subjects of the district government (Tehran, Iran). Rats were housed at a temperature of 20°C environment and exposed to alternate light and dark cycles, 12h each. Subjects were randomly divided into different experimental groups prior to the operation procedure (see below). Sham operated receiving 10 µl normal saline was infused icv in each ventricle on days 1 and 3 (n=8); control group receiving 10 µl stz (3 mg/kg) via ivc in each ventricle on days 1 and 3 (stz, n=8); group A, after one day receiving stz (3 mg/kg via

ivc), animals were treated with 1 mg/kg (iv) tac-loaded chitosan (stz+tac-chitosan (n=8); group B, after one day receiving stz (3 mg/kg via ivc), animals were treated with 1 mg/kg (iv) tac-loaded magnetic chitosan and a magnet (4000 Gaussauss/cm strength) was placed on the head for 60 min (stz+tac-mchitosan, n=8)

Surgical procedure

Rats were deeply anesthetized with ketamine (100 mg/kg) and xylazine (25 mg/kg) (both Razi Co., Iran) intraperitoneally. For chronic implantation of cannula (21 g) into lateral cerebral ventricles (icv) under stereotactic guidance, appropriate narcosis was verified by reflex testing such as the lack of ocular reflex and the absence of a pedal withdrawal response to a hard pinch. Therefore, animals were fixed in a stereotactic apparatus, and a midline incision was made in the skin. Then, a small hole was induced in the cranial region and the guide cannula was implanted and fixed. Coordinates were AP −0.8 mm, L ±1.5 mm (midline) and 3.6 mm deep from the dura. Animals were allowed one day to recover before treatment. Stz and its carrier were administered at day 1 and 3 after surgery with a 10 µl Hamilton syringe (10 µl at a rate of 1 µl/min) connected via a Teflon tube to an injector that exceeded by 2 mm the length of the guide cannula [4,6].

Intravenous administration

Prior to intravenous administration of the drug, animals were anesthetized with isoflurane and positioned laterally. Single doses of magnetic chitosan, chitosan plus tac (1 mg/kg), or magnetic chitosan plus tac were injected via the tail vein at day 1.

Morris water maze test

Spatial learning and memory of animals were tested in a Morris water maze [21]. It consisted of a circular water tank (160 cm diameter, 60 cm height) filled with water (25±1°C) to a depth of 25 cm. A non-toxic water dispersible emulsion was used to render the water opaque. Four equally spaced locations around the edge of the pool (North, South, East, and West) were used as start points which divided the pool into 4 quadrants. An escape platform (10 cm in diameter) was placed in the pool 2 cm below the surface of water. The escape platform was placed in the middle of one of the randomly selected quadrants of the pool and kept in the same position throughout the entire experiment (north-east for this study). Before training started, rats were allowed to swim freely into the pool for 90 s without platform.

Animals received a training session consisting of 4 trials per session (once from each starting point) for 4 days (days 14, 15, 16 and 17), each trial having a ceiling time of 90 s and a trial interval of approximately 30 s. After climbing onto the hidden platform, animals remained there for 30 s before commencement of the next trial. If the rat failed to locate the hidden platform within the maximum time of 90 s, it was gently placed on the platform and allowed to remain there for the same interval of time. The time taken to locate the hidden platform (latency in s) was measured.

Twenty four hours after the acquisition phase, a probe test (day 17) was conducted by removing the platform. Rats were allowed to swim freely in the pool for 90 s and the time spent in target quadrant, which had previously contained the hidden platform, was recorded. The time spent in the target quadrant indicated the degree of memory consolidation which had taken place after learning.

RNA isolation and real time RT-PCR

After removing the brain, the entire dorsal and ventral hippocampal formation including Ammon´s horn, dentate gyrus, and subiculum was dissected following the removal of the basal hypothalamus (Ivanova and Beyer, 2000). Tissue pieces were immediately frozen in liquid nitrogen and maintained at -70°C until RNA isolation.

After tissue homogenization, total RNA was isolated using the Trizol reagent (GIBCO BRL Life Technologies, Gaithersburg, USA) according to the manufacturer's recommended protocol. RNA samples were reverse-transcribed by Moloney murine leukemia virus (MMLV) reverse transcriptase (Superscript RT GIBCO, D) and oligo-(dT)15 primers (Promega, USA). Subsequently, SYBR Green Master PCR Mix (Applied Biosystems) and target specific PCR primers for seladin-1 for 5'-GGGTGTTTGTGTGCCTCTTCC and rev 3'-GCTCCTTCCACTCCCGTACC and amyloid precursor protein (APP) for 5'-GGCCCTCGAGAATTACATCA and rev 3'-GTTCATGCGCTCGTAGATCA were used for amplification of cDNA samples by using real time quantitative PCR equipment (7500 Fast Real Time PCR System, Applied Biosystems) applying a standardized protocol. Relative quantification was performed using the deltaCt method which results in ratios between target genes and a housekeeping reference gene (22). Gene expression was monitored using the ABI Prism 7000 apparatus (Applied Biosystems, USA). For data calculation, values of saline-treated animals were set to 1.

Statistical analysis

All results represent means±SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test with $p \leq 0.05$ as statistically significant

RESULTS

Behavioral studies

The Morris water maze test was performed to examine hippocampal-dependent spatial learning and memory. As shown figures 1A and B, spatial memory testing in rats receiving stz was significantly reduced ($p \leq 0.001$) suggesting that AD-like symptoms have been successfully triggered.

In the spatial version of the water maze test, the swimming speed did not differ between normal saline, stz, stz+tac-loaded magnetic and non-magnetic chitosan and stz+magnetic chitosan and non-magnetic chitosan groups (data are not shown). During the five days of training, tac-loaded magnetic chitosan and non-magnetic chitosan exposed rats significantly improved their performance as shown by reduced escape latency to reaching the platform (and also shortened distance) ($p \leq 0.001$) compared to the stz group (figure 1A). However, the average latency stz rats needed to find the hidden platform was significantly longer ($p \leq 0.001$) than the saline group, indicating that these rats revealed deficits in spatial learning and memory. The groups receiving stz plus tac-loaded magnetic and non-magnetic chitosan (stz+tac+Ch, stz+tac+mCh) did not show significant differences compared to the normal saline group but were significantly reduced compared to the stz group ($p \leq 0.0001$) suggesting a beneficial effect of tac-loaded chitosan. During the trials where rats tried to find the hidden platform, tac-loaded magnetic and non-magnetic chitosan rats spent more time in the target area than in any of the other three quadrants ($p \leq 0.001$). In contrast, stz rats spent ~30% of the time in the target quadrant which was not significantly different from the 25% chance level (figure 1C). In the non-spatial version of the water maze test, stz and groups with tac-loaded magnetic and non-magnetic chitosan and saline rats performed equally in locating a cued-platform ($p=0.98$)

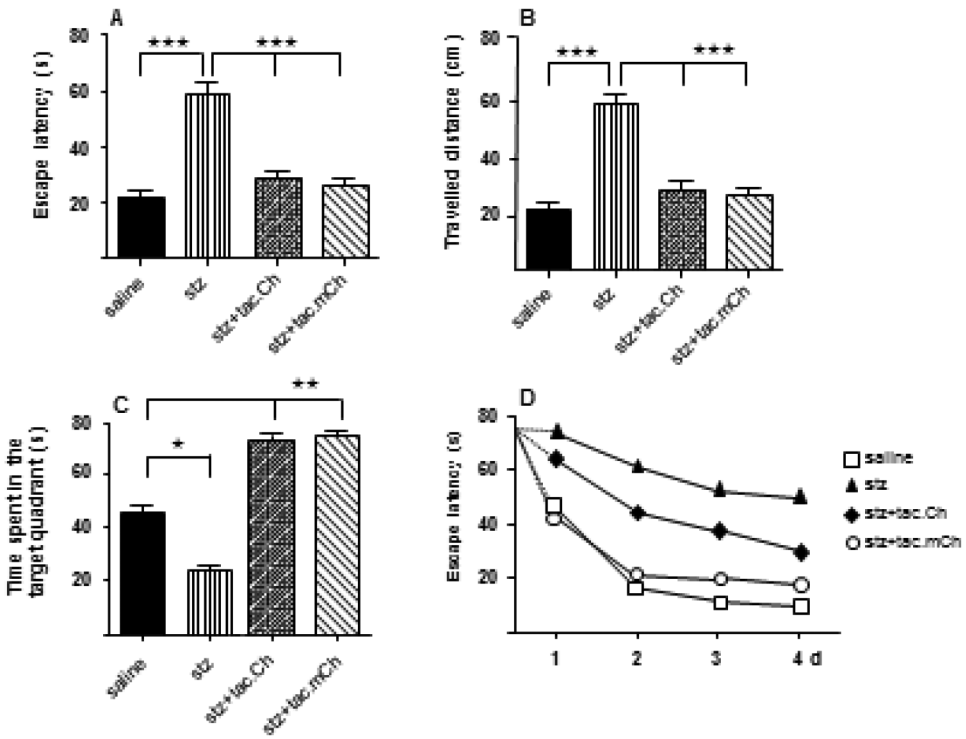

Figure 1. Effect of treatment on behavioral performance. (A) Escape latency and (B) travelled distance both after 4d, (C) time spent in the target quadrant after 4d, (D) escape latency during 4d training period. Stz increased escape latency and travelled distance compared to saline control animals (***$p \leq 0.0001$ stz vs saline). These effects were antagonized by the co-application of tac+Ch and tac+mCh at equal levels (***$p \leq 0.0001$). In contrast, stz reduced the time spent in the target quadrant (*$p \leq 0.01$ stz vs saline). Again, this effect was abolished by either of the two above treatment groups. Moreover, all two groups show increased levels compared to the saline group (**$p \leq 0.001$). Time course of escape latency experiments expressed from an individual animal per group (D) reveals that in all groups a reduction can be observed during the training period with worst improvement in the stz group and equal learning improvement in the mCH and saline group. The initial slope of acquisition was much faster in the saline and mCh group.

which indicates that these groups had comparable motor abilities and visual acuity. The lack of preference for the target quadrant together with the poor performance during training suggested that stz rats had impaired spatial learning and memory before the appearance of amyloid plaques. Figure 1D demonstrates that all groups showed a declining latency time to reach the escape platform during the training period with a slightly stronger slope in the stz+tac+Ch and mch groups which however only became significant between groups on day 4 (compare to data shown in figure 1A).

Expression of seladin-1 and APP in the hippocampus

The amount of seladin-1 mRNA was assessed by real-time RT-PCR (Figure 2A). Significantly ($p \leq 0.05$) reduced levels of seladin-1 transcripts were observed in the stz group in comparison to the saline group. Treatment with either tac+Ch and to a significant higher extent with tac+mCh further boosted seladin-1 expression significantly by 2- and 4-times, respectively ($p \leq 0.005$ and $p \leq 0.0001$).

APP expression was significantly ($p \leq 0.05$) increased after stz treatment compared to saline (Figure 2B). The application of tac+Ch or tac+mCh significantly ($p \leq 0.001$) prevented this effect at a similar level and further diminished APP levels to approx. 20-30% compared to saline levels.

DISCUSSION

AD is an irreversible and slowly progressing complex neurodegenerative disease of the brain [15]. This brain disease is characterized in pathophysiological terms by the presence of parenchymal Aß plaques mainly within the cerebral cortex and intra-neuronal NFT [23]. To which extent these hallmarks contribute to the resulting neurological deficits and disease progression is still a matter of controversial discussion. Nevertheless, in final disease stages a massive loss of cerebral cortex tissue and shrinkage of brain mass can be observed as a result of neuronal cell loss in the respective brain areas [8,9]. *In vitro* and *in vivo* studies have showed that AChE has the bio (chemical) property to bind to Aß senile plaques thereby further promoting its aggregation both in vitro and in vivo [24]. Furthermore, AD is paralleled by a progressive dysfunction and decline of the cholinergic neurotransmitter system in the CNS [25]. These observations make it plausible that pharmaceutical approaches tackling the cholinergic brain system might have beneficial effects on disease progression and might slow down symptoms [24,25]. The use of AChE inhibitors may therefore result in benefits of patients [24] and it was suggested to improve memory function [26]. Nevertheless, there is still some controversy about using AChE inhibitors in long-term treatment [24]. Among AChE inhibitors, tac was the first drug approved for AD by the U.S. Food and Drug Administration (FDA) [27]. Several clinical trials have supported the effectiveness of tac in AD with respect to delay the onset and severity of symptoms, although this compound cannot stop or cure AD [12]. However to obtain sufficient efficacy, high doses of tac are required which may cause side effects such as gastrointestinal problems [18]. Therefore, a target-specific release to and an accumulation within the brain structures of interest would

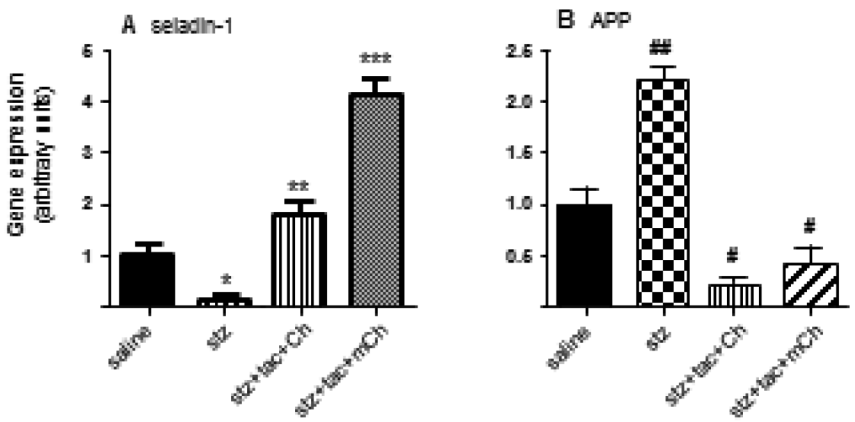

Figure 2. Effect of treatment on seladin-1 (A) and APP (B) expression analyzed by real-time PCR. Stz application reduced seladin-1 significantly ($p \leq 0.05$). This effect was counteracted by the co-application of tac-loaded chitosan. The use of magnetic chitosan further boosted tac effects. Stz application increased APP expression. Here, both non-magnetic and magnetic chitosan plus tac completely prevented this effect and further reduced APP levels significantly vs the saline group. *$p \leq 0.05$ stz vs saline, **$p \leq 0.005$ stz+tac+Ch vs stz or vs. saline, ***$p \leq 0.0001$ stz+tac+mCh vs stz or vs. saline, #$p \leq 0.001$ stz+tac+Ch/stz+tac+mCh vs stz, ##$p \leq 0.05$ stz vs saline. Data for saline treatment were set to 1.

be of therapeutic advantage to minimize non-target effects. Special attention has been given to the development of novel and effective drug delivery systems that are capable of carrying the drug and providing sufficient bioavailability in the CNS. In particular, the field of nano-neurotechnology, i.e. the application of nanoparticles for target-specific drug delivery, has developed during the past years [27]. One of the major problem of these technology is the controlled drug release at the target site(19). The use of externally controlled delivery systems such as magnetic tools could help to overcome some problems [20]. Magnetically-targeted delivery systems are promising and allow to accumulate the therapeutic drug within the target area by improving the microvascular permeability and interstitial penetration [28].

Our study was undertaken to analyze the effect of tac-loaded to magnetic and non-magnetic chitosan micro-particles on recovery and/or prevention of AD symptoms in rats achieved by treating the animals with stz. In a previous study, tac accumulation in the liver and spleen was reduced after coating nanoparticles with 1% polysorbate 80 [29]. In our set-up, micro-particles were injected iv and a suitable magnet were place over the skull of the animals. Chitosan nanoparticles are considered as suitable tools to be used for drug delivery in medical therapy [29]. For our purpose, magnetic particles were incorporated in chitosan polymers. This modification leads to a retention of the magnetic carrier at the target size, delayed reticulo-endothelial clearance, and facilitated extravasations, thereby causing prolonged activity of the applied drug [30]. We used the intracerebroventricular administration of stz to obtain AD-like long-term symptoms in rats such as learning and memory disabilities [30]. We could clearly demonstrate that stz application causes a reliable aggravation of behavioral deficits. The treatment with tac-loaded non-magnetic and magnetic chitosan prevented behavioral decline with respect to escape latency, travelled distance, and time spent in the target quadrant to a similar extent. With respect to levels of toxic APP [32] and neuroprotective seladin-1 levels which confers resistance to AD-associated neurodegeneration and oxidative [33], we found that in particular the magnetic approach induced seladin-1 expression, whereas both forms of chitosan particles were equally effective in preventing APP expression. Although in our behavioral studies both particle forms were similarly successful and only seladin-1 expression was measurable increased by the application of the magnetic chitosan nanoparticles, we assume that in AD models, the magnetic approach is promising for future studies. This is also suggested by others studies where the tac concentration could be significantly increased in the target brain region by the use of an magnetic experimental set-up in AD models [30].

Altogether, we assume that the chosen magnetic delivery approach using nanoparticles is a new promising tool for the selective treatment of brain diseases, since these particles easily cross the blood-brain-barrier and can therefore be selectively released and display their highest bioactivity at the target site of action [29,34] .

REFERENCE

1. Lim YY, Maruff P, Schindler R, Ott BR, Salloway S, Yoo DC, et al. Disruption of cholinergic neurotransmission exacerbates Aβ-related cognitive impairment in preclinical Alzheimer's disease. Neurobiology of Aging. 2015.
2. Ramirez MJ, Lai MK, Tordera RM, Francis PT. Serotonergic therapies for cognitive symptoms in Alzheimer's disease: rationale and current status. Drugs. 2014;74(7):729-36.
3. Benhamú B, Martín-Fontecha M, Vázquez-Villa H, Pardo L, López-Rodríguez ML. Serotonin 5-HT6 receptor antagonists for the treatment of cognitive deficiency in Alzheimer's disease. Journal of medicinal chemistry. 2014;57(17):7160-81.
4. Hassanzadeh G, Hosseini A, Pasbakhsh P, Akbari M, Ghaffarpour M, Takzare N, et al. Trimetazidine Prevents Oxidative Changes Induced in a Rat Model of Sporadic Type of Alzheimer´ s Disease. Acta Med Iran. 2015;53(1):17-24.
5. Milovanovic M, Eriksson K, Winblad B, Nilsson S, Lindahl TL, Post C, et al. Alzheimer and platelets: low-density platelet populations reveal increased serotonin content in Alzheimer type dementia. Clinical biochemistry. 2014;47(15):51-3.
6. Alipour F, Oryan S, Sharifzadeh M, Karimzadeh F, Kafami L, Irannejad H, et al. The Neuroprotective Effect of a Triazine Derivative in an Alzheimer's Rat Model. Acta Med Iran. 2014;53(1):8-16.
7. Goure WF, Krafft GA, Jerecic J, Hefti F. Targeting the proper amyloid-beta neuronal toxins: a path forward for Alzheimer's disease immunotherapeutics. Alzheimers Res Ther. 2014;6(4):42.
8. Folch J, Junyent F, Verdaguer E, Auladell C, Pizarro JG, Beas-Zarate C, et al. Role of cell cycle re-entry in neurons: a common apoptotic mechanism of neuronal cell death. Neurotoxicity research. 2012;22(3):195-207.
9. Duan L, Bhattacharyya BJ, Belmadani A, Pan L, Miller RJ, Kessler JA. Stem cell derived basal forebrain cholinergic neurons from Alzheimer's disease patients are more susceptible to cell death. Mol Neurodegener. 2014;9(3).
10. Schliebs R, Arendt T. The cholinergic system in aging and neuronal degeneration. Behavioural brain research. 2011;221(2):555-63.
11. Costa RO, Ferreiro E, Oliveira CR, Pereira CM. Inhibition of mitochondrial cytochrome c oxidase potentiates Aβ-induced ER stress and cell death in cortical neurons. Molecular and Cellular Neuroscience. 2013;52:1-8.

12. Antequera D, Bolos M, Spuch C, Pascual C, Ferrer I, Fernandez-Bachiller MI, et al. Effects of a tacrine-8-hydroxyquinoline hybrid (IQM-622) on Aβ accumulation and cell death: involvement in hippocampal neuronal loss in Alzheimer's disease. Neurobiology of disease. 2012;46(3):682-91.
13. Um H-S, Kang E-B, Koo J-H, Kim H-T, Kim E-J, Yang C-H, et al. Treadmill exercise represses neuronal cell death in an aged transgenic mouse model of Alzheimer's disease. Neuroscience research. 2011;69(2):161-73.
14. Danysz W, Parsons CG. Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine–searching for the connections. British journal of pharmacology. 2012;167(2):324-52.
15. Tsai GE, Falk WE, Gunther J, Coyle JT. Improved cognition in Alzheimer's disease with short-term D-cycloserine treatment. 2014.
16. Mattson MP. Pathways towards and away from Alzheimer's disease. Nature. 2004;430(7000):631-9.
17. Fernández-Bachiller MaI, Pérez C, González-Munoz GC, Conde S, López MG, Villarroya M, et al. Novel Tacrine–8-hydroxyquinoline hybrids as multifunctional agents for the treatment of alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper-complexing properties. Journal of medicinal chemistry. 2010;53(13):4927-37.
18. Birks J. Cholinesterase inhibitors for Alzheimer's disease (Review). 2012.
19. Bae KH, Park M, Do MJ, Lee N, Ryu JH, Kim GW, et al. Chitosan oligosaccharide-stabilized ferrimagnetic iron oxide nanocubes for magnetically modulated cancer hyperthermia. ACS nano. 2012;6(6):5266-73.
20. Häfeli U. Magnetically modulated therapeutic systems. International journal of pharmaceutics. 2004;277(1):19-24.
21. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature protocols. 2006;1(2):848-58.
22. Schonrock N, Ke YD, Humphreys D, Staufenbiel M, Ittner LM, Preiss T, et al. Neuronal microRNA deregulation in response to Alzheimer's disease amyloid-beta. PloS one. 2010;5(6):e11070.
23. Okamura N, Furumoto S, Fodero-Tavoletti MT, Mulligan RS, Harada R, Yates P, et al. Non-invasive assessment of Alzheimer's disease neurofibrillary pathology using 18F-THK5105 PET. Brain. 2014:awu064.
24. Brousseau G, Rourke BP, Burke B. Acetylcholinesterase inhibitors, neuropsychiatric symptoms, and Alzheimer's disease subtypes: An alternate hypothesis to global cognitive enhancement. Experimental and clinical psychopharmacology. 2007;15(6):546.
25. Holzgrabe U, Kapková P, Alptüzün V, Scheiber J, Kugelmann E. Targeting acetylcholinesterase to treat neurodegeneration. 2007.
26. Pepeu G, Giovannini MG. Cholinesterase inhibitors and memory. Chemico-biological interactions. 2010;187(1):403-8.
27. Suh WH, Suslick KS, Suh Y-H. Therapeutic agents for Alzheimer's disease. Current Medicinal Chemistry-Central Nervous System Agents. 2005;5(4):259-69.
28. Wilson B, Samanta MK, Santhi K, Kumar KS, Ramasamy M, Suresh B. Chitosan nanoparticles as a new delivery system for the anti-Alzheimer drug tacrine. Nanomedicine: Nanotechnology, Biology and Medicine. 2010;6(1):144-52.
29. Yuan X-b, Li H, Yuan Y-b. Preparation of cholesterol-modified chitosan self-aggregated nanoparticles for delivery of drugs to ocular surface. Carbohydrate Polymers. 2006;65(3):337-45.
30. Wilson B, Samanta MK, Santhi K, Kumar KPS, Ramasamy M, Suresh B. Significant delivery of tacrine into the brain using magnetic chitosan microparticles for treating Alzheimer's disease. Journal of neuroscience methods. 2009;177(2):427-33.
31. Khan MB, Ahmad M, Ahmad S, Ishrat T, Vaibhav K, Khuwaja G, et al. Bacopa monniera ameliorates cognitive impairment and neurodegeneration induced by intracerebroventricular-streptozotocin in rat: behavioral, biochemical, immunohistochemical and histopathological evidences. Metabolic brain disease. 2015;30(1):115-27.
32. Roher AE, Esh CL, Kokjohn TA, Castaño EM, Van Vickle GD, Kalback WM, et al. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. Alzheimer's & Dementia. 2009;5(1):18-29.
33. Behl C. Brain aging and late-onset Alzheimer's disease: many open questions. International Psychogeriatrics. 2012;24(S1):S3-S9.
34. Lien C-F, Molnár Ev, Toman P, Tsibouklis J, Pilkington GJ, Górecki DC, et al. In vitro assessment of alkylglyceryl-functionalized chitosan nanoparticles as permeating vectors for the blood–brain barrier. Biomacromolecules. 2012;13(4):1067-73.