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Possible involvement of PI3K/AKT/mTOR signaling pathway in the protective effect of selegiline (deprenyl) against memory impairment following ischemia reperfusion in rat

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ABSTRACT

Short-term cerebral ischemia led to memory dysfunction. There is a pressing need to introduce effective agents to reduce complications of the ischemia. Involvement of PI3K/AKT/mTOR signaling pathway has been determined in the neuroprotective effect of various agents. Selegiline (deprenyl) possessed neuroprotective properties. In this study global ischemia/reperfusion was established in rats. Selegiline (5 mg/kg for 7 consecutive days) administrated via intraperitoneal route. Possible involvement of PI3K/AKT/mTOR signaling pathway was evaluated using qRT-PCR, immunohistochemistry and histophatologic evaluations in the hippocampus. Spatial memory was evaluated by morris water maze (MWM). Results showed that ischemia impaired the memory and ischemic rats spent more time to find hidden platform in the MWM. Ischemia significantly decreased levels of PI3K, AKT and mTOR in the hippocampus. Histopathologic assessment revealed that the percent of dark neurons significantly increased in the CA1 area of the hippocampus of ischemic rats. Selegiline improved the memory as ischemic rats spent fewer time to find hidden platform in the MWM. Findings showed that selegiline increased the level and expression of PI3K, AKT and mTOR as well as decreased the proportion of dark neurons in the CA1 area of the pyramidal layer of the hippocampus. We concluded that selegiline, partially at least, through increases the expression of PI3K, AKT and mTOR as well as decreases the percent of dark neurons in the hippocampus could improve the memory impairment following the ischemia in rats.

1. Introduction

Stroke is one of the most common causes of disability with high economic burden and increasing incidence in the world (Mozaffarian et al., 2016; Hirt et al., 2017; Schuhmann et al., 2017). Cut of blood flow to the brain in the ischemic stroke (IS) is associated with the brain injury (Bi et al., 2017; Jia et al., 2008). Short-term cerebral ischemia led to neuronal necrosis, apoptotic cell death, silent infarcts and cognitive decline (Ünal et al., 2001). Several clinical and preclinical studies have demonstrated that ischemic stroke led to memory dysfunction, neurodegeneration and cognition impairment (Schaapsmeerders et al., 2015; Eve et al., 2016; Silva et al., 2015; Sadelli et al., 2017). Although several agents have been introduced for treatment of stroke, little have effectiveness in this disorder (O'collins et al., 2006; Sacco et al., 2007). Today, thrombolysis is only acute treatment available apply to restore blood flow to the ischemic area. In this regards, tissue plasminogen activator (tPA) approved for acute treatment. Unfortunately, tPA has some adverse effects including hemorrhage and also has short therapeutic time-window (Siket, 2016; Karatas et al., 2018). Indeed, evaluation and development of novel agents with high therapeutic index and protective effects on memory impairment consequence of ischemis warranted further studies.

Selegiline (deprenyl) is a selective and irreversible inhibitor of the monoamine oxidase (MAO)-B broadly administrated for Parkinsonism patients (Mizuno et al., 2017; Cereda et al., 2017). It has been showed that selegiline at higher doses acts as non-selective inhibitor of MAO-A and MAO-B enzymes so is effective for treatment of major and atypical depression (Finberg and Tenne, 1982; Youdim and Weinstock, 2004; Youdim and Bakhle, 2006). In case of preclinical studies, literature demonstrated that selegiline improves motivational dysfunctions and

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also exerts antidepressant effect (Yohn et al., 2017; Contreras-Mora et al., 2018; Amiri et al., 2016). Selegiline enhances striatal dopamine concentrations and has amphetamine-like action in the brain (Lamensdorf et al., 1996; Reynolds et al., 1978; Kalász et al., 2014). It has been well-known that levels of dopamine significantly decreased in Alzheimer's disease (AD). In this concept, studies have clarified that augmentation of dopaminergic activity improve memory and learning deficit in animal model of AD (Golani et al., 2014; Okada et al., 2015; Kemppainen et al., 2015; Martorana and Koch, 2014). It has been determined that acute and chronic administration of selegiline possessed anti-apoptotic and neuroprotective effects and reduce the size of infarct area in experimental ischemia (Semkova et al., 1996; Ünal et al., 2001). An explorative study showed that l-deprenyl significantly improves cognitive tests and functional recovery in stoke patients (Bartolo et al., 2015). However, the exact mechanisms of protective effect of selegiline in IS are still unknown.

The phosphatidylinositol 3-kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway is an important intracellular cascade controls cell proliferation, differentiation, cellular metabolism, apoptosis, cell survival and cytoskeletal restructuring (Janku et al., 2012; Polivka and Janku, 2014; Porta et al., 2014; Peltier et al., 2007). Previous studies showed that mTOR/cadherin signaling is involved in cell growth and adhesion (Wei and Wang, 2018; Jiang et al., 2018; Yin et al., 2018). It has been demonstrate that PI3K/AKT has a pivotal role in the proliferation of hippocampal neural progenitor cells (Peltier et al., 2007). Activation of PI3K/AKT cascade triggers neural stem cells proliferation consequently induced neurogenesis (Le Belle et al., 2011). The PI3K/AKT/mTOR pathway exerted neuroprotive activity in traumatic brain injury. In this regards, it has been determined that this pathway via suppression of neuronic autophagy in the hippocampus, possessed neuroprotective effects (Zhang et al., 2017). Researchers showed that activation of AKT/mTOR pathway possessed neuroprotective effects in ischemic brain injury (Huang et al., 2014). Recently, it has been well-known that activation of PI3K/AKT/mTOR pathway exerted the neuroprotective effect via decrease of oxidative stress, improvement of neurotransmission and neurogenesis in the AD induced by Amyloid-β in rat (Singh et al., 2017). However, there is currently almost no data about involvement of this signaling pathway in the protective effect of selegiline.

Since ischemia accounts for majority of strokes, it is crucial to evaluate the underlying mechanisms of cerebral ischemia. Therefore, introducing effective therapeutic targets has high importance to prevent neural damage in ischemic injuries of the brain. Considering neuroprotective effect of PI3K/AKT/mTOR signaling pathway and also above-mentioned beneficial effects of selegiline in ischemia, in the current study we aimed to evaluate the possible involvement of PI3K/AKT/mTOR pathway in advantageous effect of selegiline (L-deprenyl) in rat model of stroke.

2. Materials and methods

2.1. Animals

Forty male, two months old Sprague Dawley rats (Pasteur institute, Tehran, Iran) weighing 250–300 g were used. Animals were kept in Plexiglas boxes under standard laboratory conditions (temperature: 22 ± 2 °C, humidity: 50 ± 10%, 12-h light–dark cycle and free access to food and water ad libitum). All procedures were performed according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication # 80–23) and institutional guidelines for animal care and use (Shahrekord University of Medical Sciences. Shahrekord, Iran). Each experimental group contained 10 animals.

2.2. Study design

Selegiline HCl (Sigma, St Louis, MO, USA) was dissolved in saline and injected subcutaneously (s.c.) at dose of 5 mg/kg for 7 consecutive days. Dose and duration of selegiline's administration was selected according to previous published studies (Amiri et al., 2016; Tsunekawa et al., 2018; Shimazu et al., 2005) and our pilot studies.

Of the forty rats used in this study, twenty rats were subjected to global ischemia model and twenty rats were remained intact. Rats were divided into four groups as follows: 1) Control group without surgery received saline 2) rats which were underwent ischemia reperfusion model and received saline 3) ischemic reperfusion rats received selegiline and 4) rats which were underwent ischemia reperfusion model and received selegiline.

Rats were treated with saline or selegiline for 7 days (days 0–7) and then were subjected to water maze test for evaluation of memory. After memory assessment, rats were euthanized under anesthesia using pentobarbital (60 mg/kg,i.p.) and hippocampi were dissected out and histopathological changes in the CA1 area as well as expression of PI3K, AKT and mTOR genes were evaluated in the hippocampus using RT-PCR method. In addition, the level of PI3K, AKT, mTOR and p-mTOR (phosphorylated mTOR) was evaluated by immunohistochemistry method.

2.3. Global ischemia/reperfusion model establishment

Transient global ischemia was induced according to the previously described method (Li et al., 2006; Cao et al., 2011). For short, anesthesia was induced by intraperitoneal administration of ketamine (60 mg/kg) and xylazine (6 mg/kg). The bilateral common carotid arteries were exposed through a 2 cm ventral midline cervical opening and carefully detached from the vagus nerves, then obstructed bilaterally for 5 min using clip. Five minutes later, the clips were removed to restore cerebral blood flow and reperfusion. Animals were recovered on a heating pad for 2 h to protect from hypothermia. Also, full efforts were made to minimize the use of animals and to optimize their comfort.

2.4. Morris water maze test (MWM)

MWM is a valid device to evaluate spatial memory in rodents. The apparatus is a round black-painted tank (150 cm diameter and 60 cm deep) which filled with water (20 ± 2 °C) to a depth of 30 cm. Several distal visual objects were placed on the walls of the MWM room and their location stayed unchanged during the tests. The maze was divided into four s quadrants with four starting locations called north (N), east (E), south (S), and west (W) at same distances to the border. A Plexiglas escape circular platform (10 cm in diameter) was kept 1 cm beneath the surface of the water in the center of the north-west quadrant (target quadrant). Throughout the tests, the animal motion was recorded by a camera located above the maze which was connected to a computer. A videotracking system (Etho-Vision XT® v 8.5; Noldus Information Technology, Wageningen, the Netherlands) was used to record the time spent to find the hidden platform (escape latency) and also path length to reach the hidden platform (traveled distance). To do this experiment, rats were trained in the MWM. For this purpose, each rat was allowed to swim during 60 s to discover the hidden platform directed by distal spatial indications.

Subsequently finding the platform, animals were permitted to stay there for 20 s, and were then placed in a cage for 20 s till the start of the next trial. If an animal did not find the platform within this period, it was manually guided to the platform by the experimenter and allowed to rest for 20 s. Escape latency was recorded in each trial for evaluation of spatial memory. Probe trial (retrieval test session) was performed 24 h afterward training. The probe trial was involved a 60-s free swimming period without a platform and escape latency as well as

Table 1
Primer sequences for qRT-PCR.

Primer name	Forward sequence	Reverse sequence
AKT	TAGCCATTGTGAAGGAGGGC	CCTGAGGCCGTTCCTTGTAG
mTOR	GCTCCAGCACTATGTCACCA	CGTCTGAGCTGGAAACCAGT
Pi3K	GCAACTCCTGGACTGCAACT	CAGCGCACTGTCATGGTATG
B2m	CGTGATCTTTCTGGTGCTTGTC	GGAAGTTGGGCTTCCCATTCT

traveled distance were recorded (Amiri et al., 2016; Vorhees and Williams, 2006).

2.5. Quantitative reverse transcription–PCR (qRT-PCR)

Total RNA was extracted using Tripure isolation reagent (Roche) according to the manufacturer's instructions and quantified by a ND-100 spectrophotometer (Nanodrop Technologies). Variations in mRNA expression of looked-for genes were assessed by qRT–PCR after reverse transcription of 1 μg RNA from each sample with PrimeScript RT reagent kit (Takara) according to the manufacturer's order. The qRT–PCR was done on a light cycler apparatus (Roche Diagnostics) using SYBR Premix Ex Taq technology (Takara). Thermal cycling environment involved an initial activation phase for 30 s at 95 °C followed by 45 cycles including a denaturation step for 5 s at 95 °C and a combined annealing/extension step for 20 s at 60 °C. Beta 2-Microglobulin was considered as a normalizer and fold changes in expression of each target mRNA relative to beta 2-Microglobulin (B2m) was calculated based on $2^{-\Delta\Delta Ct}$ relative expression formula as described earlier (Haj-Mirzaian et al., 2017; Amini-Khoei et al., 2017; Lorigooini et al., 2019). The primer sequences are listed in Table 1.

2.6. Immunohistochemistry

Immunohistochemical staining was applied using the streptavidin biotin peroxidase-complex method according to our previous protocol (Sabzevary-Ghahfarokhi et al., 2018). AKT, PI3K, mTOR and p-mTOR antibodies were purchased from the Cell signaling company (Cell signaling technology, USA). In brief, hippocampi were cut into 4-μm thick sections and stuck on poly-L-lysine slides. The slides were deparaffinized and rehydrated using xylene and a series of ethanols (100%, 100%, 80% and 70%). In order to do antigen retrieving stage, sections were wrapped up in citrate buffer solution (10 mM Sodium Citrate, 0.05% Tween 20, pH 6.0) and were exposed to pressure for 20 min. To avoid nonspecific staining, slides were incubated for 2 h with protein block (Abcam, England) containing albumin. Primary antibodies were incubated overnight at 4 °C which was followed by adding 0.3% H2O2 solved in TBS to inhibit endogenous peroxidase activity. Following incubating with biotinylated IgG antibody and Streptavidin-Peroxidase Plus at room temperature, 3-diaminobenzidine tetrahydrochloride DAB was used to visualize specific antigen. Finally, Sections were counterstained with hematoxylin and washed with cool water. Intensity of immunoreactivities against primary antibodies were inspected on all sections using a light microscope (Olympus BX41) by a pathologist blind to the study using a 6-score system (0 = negative), 0.5 = 0–5% positive, 1 = 5–15% positive, 2 = 16%–40% positive, 3 = 41%–90% positive, and 5 > 90% positive.

2.7. Microscopy

After euthanasia under anesthesia using pentobarbital (60 mg/kg, i.p.), trans-cardiac perfusion was performed via 0.9% normal saline first and then continued with ice-cold 4% paraformaldehyde in 0.1Mphosphate buffer (pH 7.5). Then, the hippocampi were isolated and after fixation samples were immersed in 10% formalin. Formalin-fixed brains were paraffin-embedded and 5 μm sections were obtained. Five sections obtained from each brain and were deparaffinized using xylene and stained with H&E. Histological analysis was performed under light microscopy (400; Olympus microscope) after preparing images under objective lens using a digital camera (Olympus, Japan) and exhibited on a computer monitor. Three fields from each slide were selected and the compactness of dark neurons and normal neurons within the pyramidal cell layer of CA1 area was estimated in each field. In histological studies dark neurons are recognized by hyperbasophilia property as a type of cell degeneration. The percent of dark (dead) neurons (the relation of dark neurons to normal neurons + dark neurons (total number of neurons)) was evaluated in each group. The fields were randomly selected. All measurements were performed using Image J software by a blinded pathologist (Zsombok et al., 2005; Amini-Khoei et al., 2017).

2.8. Statistical analysis

Comparison between the groups was analyzed using two-way ANOVA followed by tukey's post test. Graph-pad prism software (version 6) was used for data analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Selegiline improved the memory function in the Morris water maze swimming test

Two- way ANOVA analysis showed that ischemic (IS) rats significantly spent fewer time in the zone1 of the apparatus in compared with control (CO) rats in the probe trail (on fifth day of test) ($P < 0.001$, Fig. 1A). Results demonstrated that following treatment with selegiline, time spent in the correct quadrant (zone1) significantly increased in the IS rats ($P < 0.001$). In case of spatial memory assessments (Fig. 1B), ischemic rats spent more time to find the escape platform in training days in comparison with control rats (training days 1 and 3 $P < 0.01$, training days 2 and 4 $P < 0.05$). Our findings showed that treatment of ischemic rats with selegiline significantly reduced the latency time to find the hidden platform in compared with saline-treated IS rats ($P < 0.05$ in training day 1 and 4).

3.2. Selegiline decreased the dead neurons (%) of the CA1 region

The percentage of dead neurons (damaged cells with sparsely arrange and fuzzy shape) were calculated inthe CA1 region of the hippocampus (Fig. 2A). The mean percentage of dead neurons in the ischemic (IS) rats was significantly higher than those in the control (CO) rats ($P < 0.001$, Fig. 2B). A significant decrease was recorded in the mean percentage of dead neurons in the selegiline-treated IS (IS + SE) rats ($P < 0.01$) in compared with the IS group.

3.3. Selegiline increased the level of AKT, PI3K, mTOR and p-mTOR in the hippocampus

As summarized in Table 2 and showed in Fig. 3 (AKT), Fig. 4 (PI3K), Fig. 5 (mTOR) and Fig. 6 (p-mTOR) ischemia hypoperfusion significantly decreased the expression of AKT, PI3K, mTOR and p-mTOR (phosphorylated mTOR) in the hippocampus in compared to the control group. Treatment with selegiline in the IS rats significantly increased the expression of AKT, PI3K, mTOR and pmTOR when compared with the saline-treated IS animals.

3.4. Selegiline increased the gene expression of AKT, PI3K and mTOR in the hippocampus

As shown in Fig. 7, expression of AKT (A), PI3K (B) and mTOR (C) was significantly decreased in the IS group in comparison with the

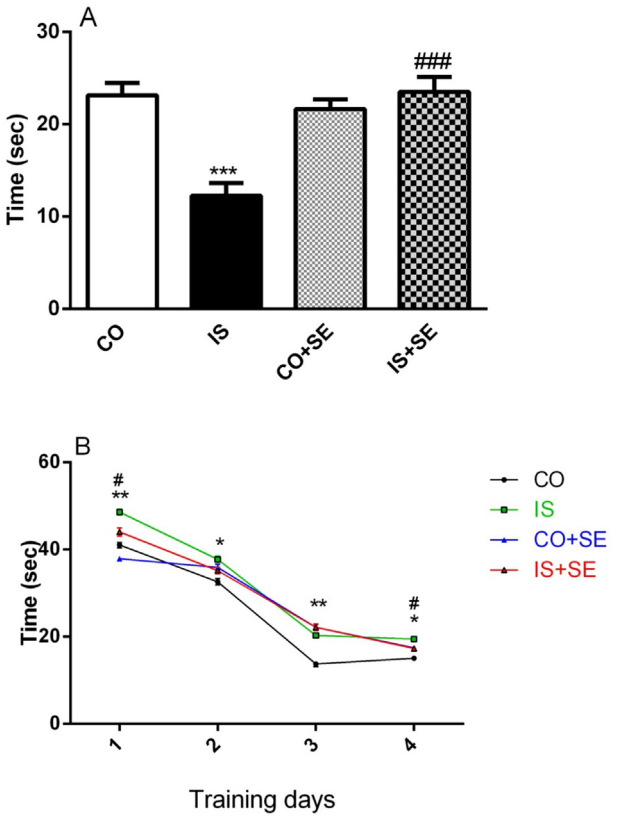

Fig. 1. (A): Spent time in zone1 through the probe trial in experimental groups. Data are presented as mean ± SD ($n = 7$). $^{***}p < 0.001$ compared with control group, $###P < 0.001$ compared with saline-treated ischemic rats. (B): Spatial learning in hidden platform in the MWM through four training days. Data are presentesd as mean ± SD ($n = 10$). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with control group, $#P < 0.05$ compared with saline-treated ischemic rats. CO (saline-treated control rat), IS (saline-treated ischemic rat), CO + SE (selegiline-treated control rat) and IS + SE (selegiline-treated ischemic rat).

control group ($P < 0.05$, $P < 0.05$ and $P < 0.001$, respectively). Furthermore, treatment with selegiline in IS rats significantly increased expression of AKT ($P < 0.01$), PI3K ($P < 0.05$) and mTOR ($P < 0.05$) in the hippocampus when compared with saline-treated IS rats.

4. Discussion

Results of the present study showed that global ischemia-reperfusion led to memory impairment status in the morris water maze test. We found that this status is accompanied with low expression of PI3K/AKT/mTOR signaling pathway at gene and protein levels as well as histopathological alterations in the hippocampus. Our findings demonstrated that treatment with selegiline reversed memory impairment following global ischemia-reperfusion model. Interestingly, this constructive behavioral effect was relevant with over expression of PI3K, AKT and mTOR as well as modification in histology of the hippocampus.

Stroke is a disabling disease with high incidence through the world which accounts for > 150 million deaths annually (Tang et al., 2012; Li et al., 2015). It has been demonstrated that cerebral ischemia is the major cause of strokes (Urban et al., 2010). Ischemic stroke is developed when cerebral blood vessel (s) is (are) occluded. Following ischemia neurons enter the apoptotic stage, initiate inflammatory responses, cell death and finally loss of brain's function is formed (Urnukhsaikhan et al., 2017; Chen et al., 2008). Following obstruction of vessels neurons encounter with an oxidative stress which result in mitochondrial dysfunction, activation of caspase family and then DNA fragmentation lastly lead to ischemic infarction in the brain (Uzar et al., 2012). Previous evaluations have been determined that subsequent of ischemia, during a period of hours or days neurons have potential to recover from injury(Moskowitz et al., 2010; Ginsberg, 2008). While there are accessible drugs for treatment of ischemic stroke but the lack of an effective treatment is felt. In addition there is no specific agent to expand functional recovery of neurons in ischemic zone. Hence, introducing a neuroprotective agent with ability for prevention of neuronal death and accelerating of recovery is needed (Ginsberg Ginsberg, 2009).

It has been demonstrated that activation of the PI3K/AKT/mTOR is important for cell proliferation and apoptosis (Annovazzi et al., 2009). Ample evidences have showed that the PI3K/AKT signaling pathway has critical role in intermediating survival signals in neurons. In this regards, it has been well-known that AKT has an anti-apoptotic role (Datta et al., 1999; Zhao et al., 2006). Beneficial effects of activation of the PI3K/AKT in neuroprotection consequence of ischemic stroke have been determined. In this concept literature said that this pathway through suppression of inflammatory response, decrease of vascular permeability and improve vascular function possessed protective effects. mTOR optimized cytotrophy, energy resource, stimulates protein synthesis and angiogenesis (Xu et al., 2008; Schabbauer et al., 2004). Considering the neuroprotective role of the PI3K/AKT/mTOR pathway in ischemic stroke we showed that this survival pathway inactivated subsequent of cerebral ischemia injury. In line with previous studies we showed that expression of PI3K/AKT/mTOR signals decreased in the hippocampus specimens of rats were subjected to ischemic stroke (Li et al., 2015). Evidences showed that activation of AKT pathway lead to activation of NF-κB transactivation resulting in initiation of transcription of survival genes such as Bcl-xL and also stimulation of trophic factors (Hussain et al., 2012; Wu et al., 2015).

Evidences demonstrated that activation of the PI3K/AKT pathway via suppression of JNK prevent neuronal cell death in cerebellar granule neurons (Choi et al., 2018; Shimoke et al., 1999). It has been determined that MTOR stimulates angiogenesis, neuronal regeneration, synaptic plasticity and removes neurotoxic substances which are linked with the recovery and survival of injured neurons in ischemic zone (Zhang et al., 2007; Chen et al., 2012a). In this concept it has been shown that inhibition of mTOR using rapamycin increased neuronal apoptosis following brain injury(Chen et al., 2012b). Our results showed that the AKT, PI3K, mTOR and p-mTOR level were significantly decreased in the hippocampus of ischemic rats. However, interestingly treatment with selegiline significantly increased the expression of aforementioned gene and proteins in the hippocampus of ischemic rats.

Selegiline [(−)-deprenyl] is an irreversible monoamine oxidase (MAO) type B inhibitor which increase level of dopamine in the striatum (Lamensdorf et al., 1996; Amiri et al., 2016). It has been resolute that selegiline metabolize to (−)-methamphetamine and (−)-amphetamine and in this way affects the brain functions (Reynolds et al., 1978). Furthermore, selegiline through upregulation of dopaminergic activity exerts beneficial effects in brain's functions such as memory and learning (Kesby et al., 2016; Kumar et al., 2018). It is well-known that agents which enhance dopaminergic neurotransmission increase activity of the PI3K/AKT/mTOR pathway (Emamian, 2012). Previous studies have demonstrated that neuroprotective properties of rasagiline in experimental model of focal ischemia were mediated through MAO independent inhibition (Speiser et al., 1999). In this regards, evidences showed that selegiline possessed neuroprotective effects and increased brain's resistance in response to ischemia (Kwon et al., 2004; Ünal et al., 2001).

CA1 pyramidal neurons are sensitive to ischemia and relatively high percentages of these neurons die following the hypoxia (Duszczyk et al., 2009). In case of learning and memory deficits, literature revealed that selegiline attenuated memory impairment following ischemic brain damage (Puurunen et al., 2001; Kesby et al., 2016). Our results showed that selegiline significantly improved memory impairment in ischemic

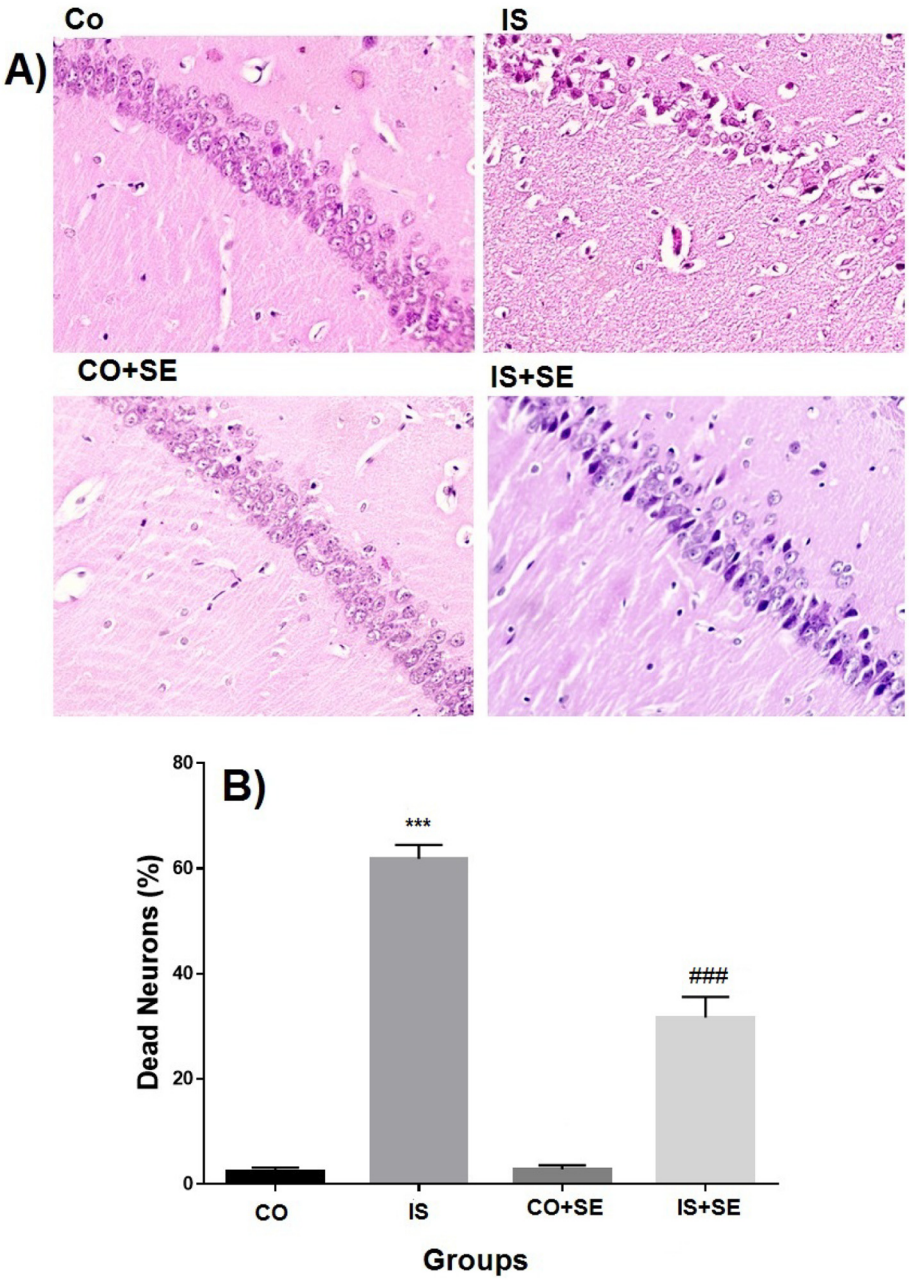

Fig. 2. The effects of treatment with selegiline on hippocampal CA1 area in hypoperfused rat, (A): Representative hematoxylin and eosin (H&E) stained slides from CA1 area (×400). (B): the percent of dead (dark) neurons in the CA1 area of the hippocampus. ***P < 0.001 compared with saline-treated control rats, ###P < 0.001 compared with the saline- treated ischemic rats.CO (saline-treated control rat), IS (saline-treated ischemic rat), CO + SE (selegiline-treated control rat) and IS + SE (selegiline-treated ischemic rat).

Table 2
Immunohistochemistry expression of AKT, PI3K, mTOR and p-mTOR in the hippocampus. The expression of AKT, PI3K, mTOR and p-mTOR were scored. Data are expressed as percent of positive cells ($n = 8$). *P < 0.05 compared with saline-treated control rats, $\#P < 0.005$ compared with the saline- treated ischemic rats. CO (saline-treated control rat), IS (saline-treated ischemic rat), CO + SE (selegiline-treated control rat) and IS + SE (selegiline-treated ischemic rat).

P-mTOR	mTOR	PIK3	AKT	Groups
14%	24%	15%	42%	CO
10%*	7%*	11%*	5% > *	IS
16%	16%	19%	38%	CO + SE
14.3%#	18.75%#	18%#	23%#	IS + SE

rats. Furthermore, following treatment with selegiline the percent of dark neurons in the CA1 area of the hippocampus significantly decreased in ischemic rats. Clinical investigations have been clarified that selegiline has therapeutic effects in Neurological diseases including Alzheimer's disease and improves cognitive impairment (Sano et al., 1997; Ebadi et al., 2006). According to experimental studies administration of selegiline enhanced the survival and density of pyramidal neurons of the hippocampus including CA1 and CA3 cells as well as decreased the proportion of dark neurons in pyramidal area (Paterson et al., 1997; Lahtinen et al., 1997).

There are evidences revealed that hippocampus is a critical area for processing of memory (Danielson et al., 2016; Garthe et al., 2016). In this regards it has been determined that expansion of connectivity and plasticity of pyramidal cell especially CA1 cells improved memory and

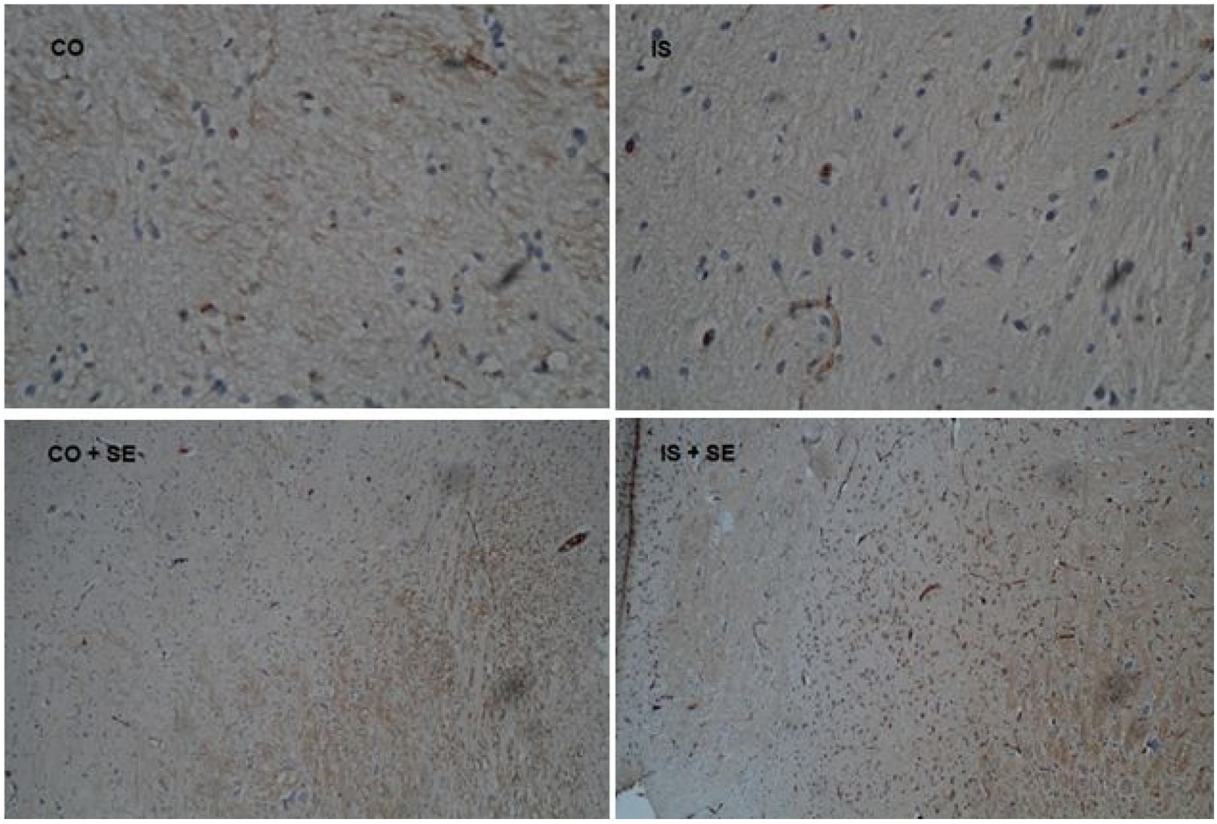

Fig. 3. The immunohistochemical features of AKT expression in the hippocampus (×400). CO (saline-treated control rat), IS (saline-treated ischemic rat), CO + SE (selegiline-treated control rat) and IS + SE (selegiline-treated ischemic rat).

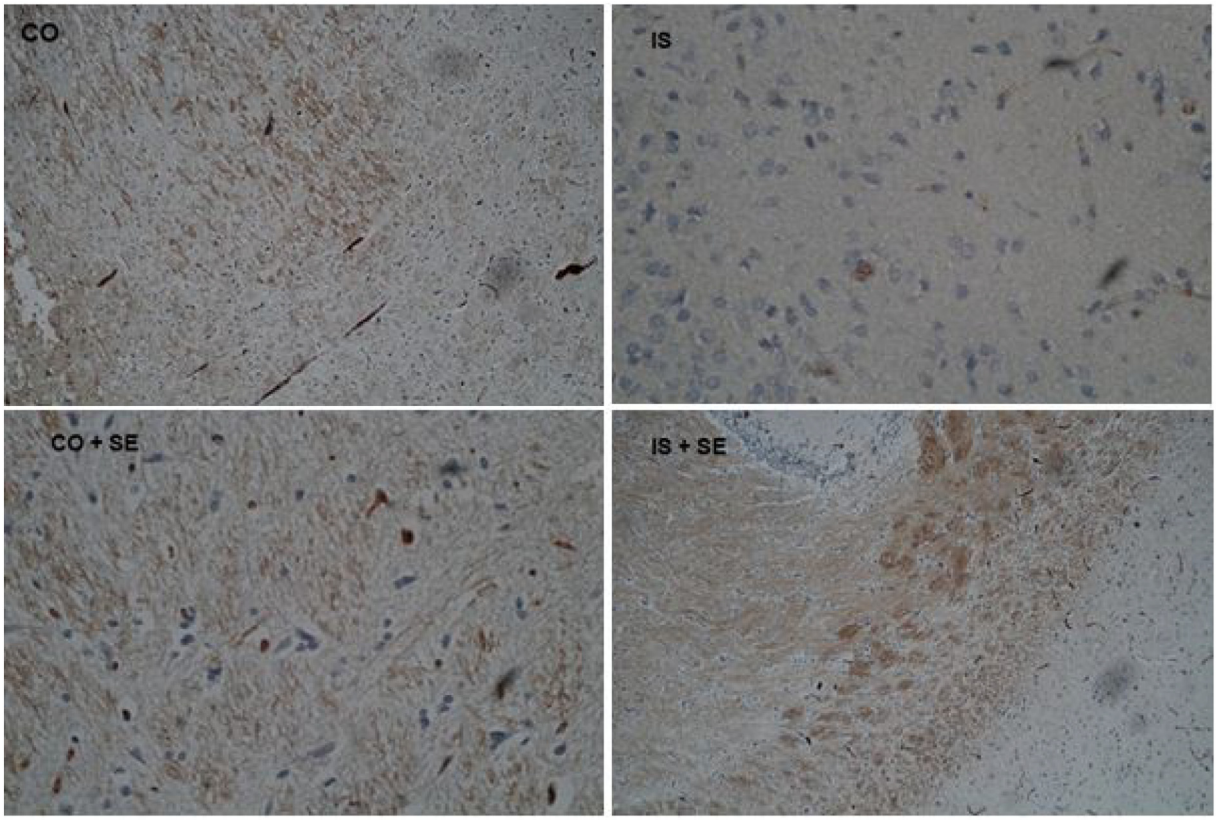

Fig. 4. The immunohistochemical features of PI3K expression in the hippocampus (×400). CO (saline-treated control rat), IS (saline-treated ischemic rat), CO + SE (selegiline-treated control rat) and IS + SE (selegiline-treated ischemic rat).

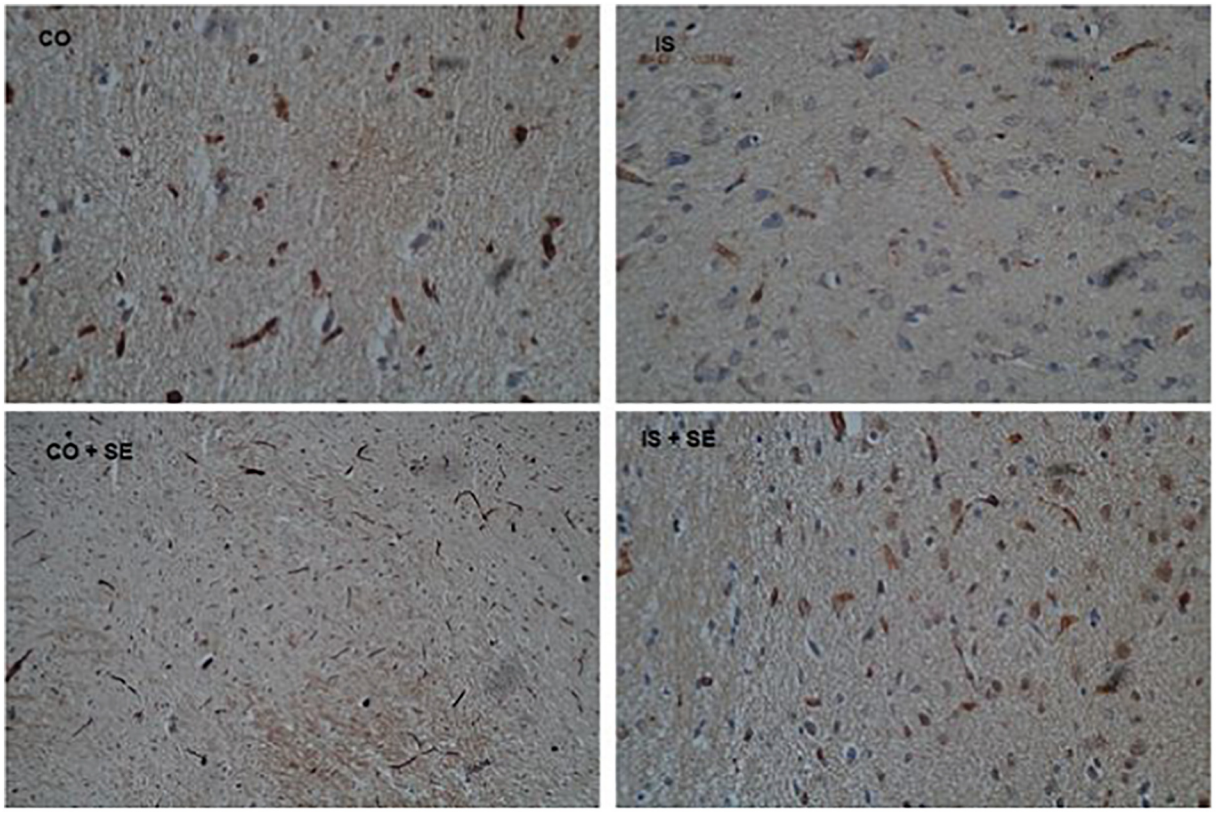

Fig. 5. The immunohistochemical features of mTOR expression in the hippocampus (×400). CO (saline-treated control rat), IS (saline-treated ischemic rat), CO + SE (selegiline-treated control rat) and IS + SE (selegiline-treated ischemic rat).

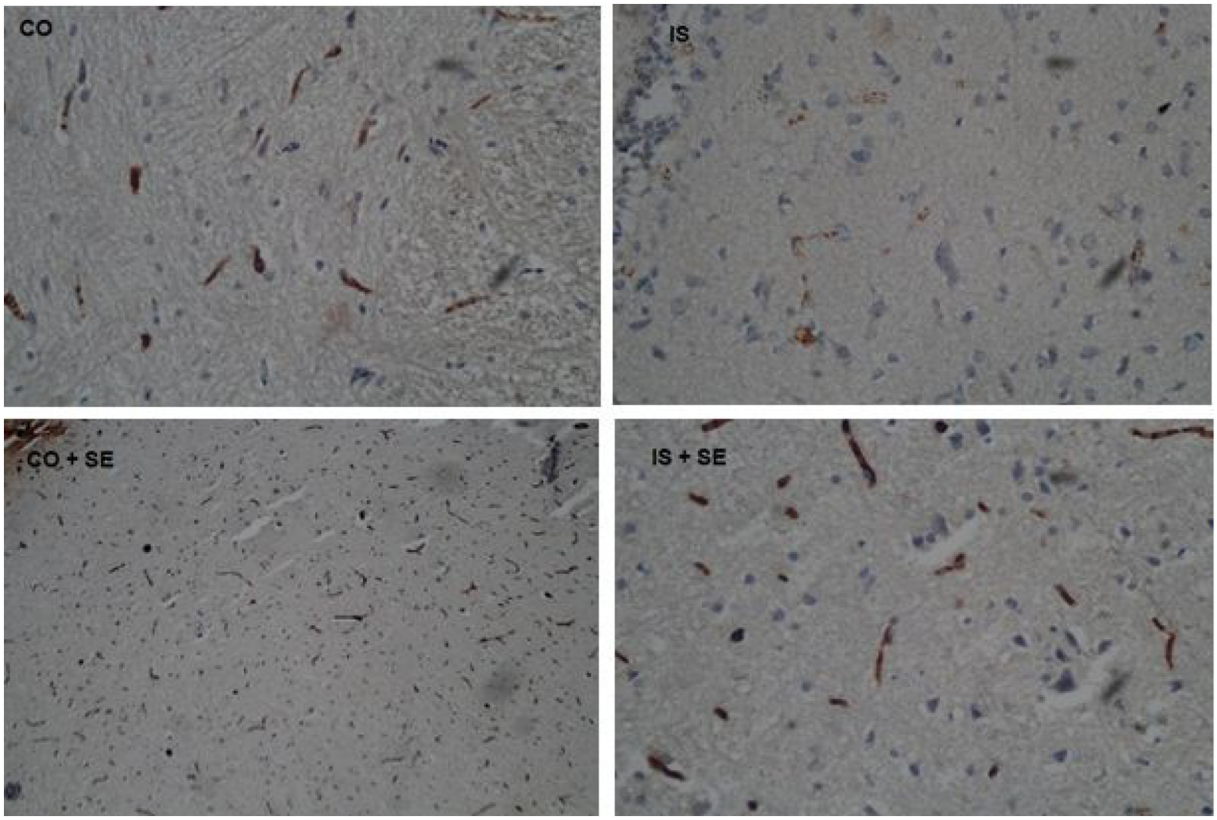

Fig. 6. The immunohistochemical features of p-mTOR expression in the hippocampus (×400). CO (saline-treated control rat), IS (saline-treated ischemic rat), CO + SE (selegiline-treated control rat) and IS + SE (selegiline-treated ischemic rat).

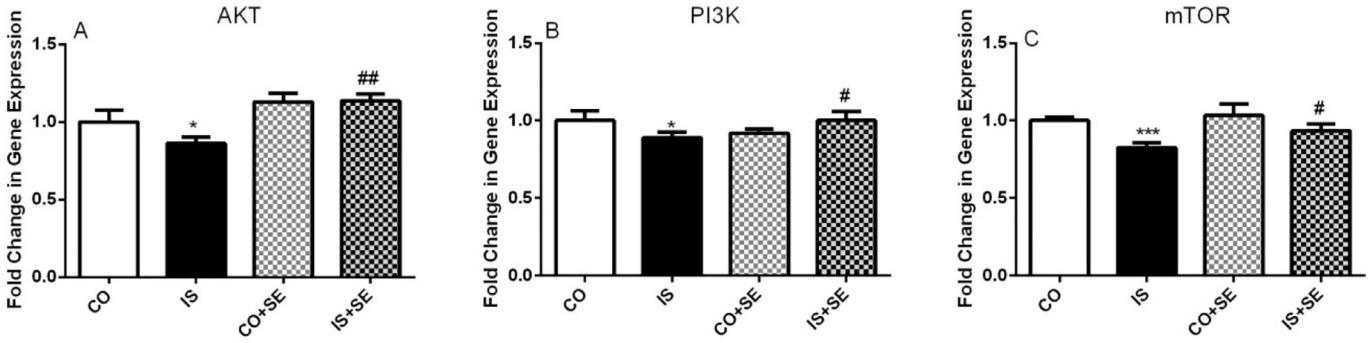

Fig. 7. The expression of AKT (A), PI3K (B) and mTOR (C) in the hippocampus was determined by qRT-PCR. Data are shown as mean ± SEM from triplicate tests and were analyzed using two- way ANOVA followed by Tukey's post-hoc test. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with saline-treated control rats, $\#P < 0.05$ and $\#\#P < 0.01$ compared with the saline- treated ischemic rats. CO (saline-treated control rat), IS (saline-treated ischemic rat), CO + SE (selegiline-treated control rat) and IS + SE (selegiline-treated ischemic rat).

learning deficits following hippocampal injury (Danielson et al., 2016; Stackman Jr et al., 2016; Havekes et al., 2016; Hansen et al., 2015).

Morris water maze as a valid behavioral test performed for evaluation of memory and learning in rodents (Wang et al., 2017). In line with previous studies we found that rats were subjected to ischemic stroke model showed memory impairment in this hippocampal-related behavioral test (Wang et al., 2017; Fan et al., 2015). Our results showed that treatment with selegiline significantly improved memory deficit in ischemic rats.

5. Conclusion

Findings of this in vivo ischemia study showed that activation of the PI3K/AKT/mTOR pathway partially, at least, has critical role in reversing the adverse impacts of ischemic model of stroke in rat. Interestingly our results showed that selegiline probably, at part, through upregulation of PI3K/AKT/mTOR in the hippocampus improves memory following ischemia in rat.

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Conflict of interests

The authors declare that there is no conflict of interest.

Compliance with ethical standards

All applicable international and institutional guidelines for the care and use of animals were followed.

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