Expression of APP, BACE1, AChE and ChAT in an AD model in rats and the effect of donepezil hydrochloride treatment

QIANG LI[1], MIN CHEN[2], HONGMIN LIU[1], LIQUN YANG[1] and GUIYING YANG[1]

[1]Nursing School and [2]Medical Technology School, Qiqihar Medical University, Qiqihar 161006, P.R. China

Received May 19, 2012; Accepted September 14, 2012

DOI: 10.3892/mmr.2012.1102

Abstract. The aim of this study was to investigate the pathological changes in a rat model of Alzheimer's disease (AD) and the effect of donepezil hydrochloride (HCl) treatment. The rat model of AD was established by the bilateral injection of amyloid β_{1-40} ($A\beta_{1-40}$) into the hippocampus. Changes in spatial learning and memory functions were examined using the Morris water maze test and changes in catalase (CAT) and glutathione peroxidase (GSH-Px) activities were determined using chemical colorimetry. Moreover, the changes in acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) expression were analyzed using immunohistochemical staining. The mRNA expression levels of the amyloid precursor protein (APP) and β-secreted enzyme 1 (BACE1) were evaluated using RT-PCR. The effects of donepezil HCl on the aforementioned indices were also observed. The rat memories of the platform quadrants in the blank, sham and donepezil HCl groups were improved compared with those of the rats in the model group. The ratio of swim distance in the fourth platform quadrant (l_4) to the total swim distance (l total) for the model group rats (l_4/l total) was significantly decreased compared with that for the blank and sham group rats. Following donepezil HCl treatment, the ratio of l_4/l total significantly increased. AD modeling caused a significant decrease in the CAT and GSH-Px activities in the brain tissues of the rats. The CAT and GSH-Px activities in the AD model rats significantly increased following donepezil HCl treatment. Moreover, donepezil HCl treatment significantly decreased the AChE, APP and BACE1 mRNA expression levels and increased the ChAT expression levels. Therefore, donepezil HCl was able to significantly decrease learning and memory damage in a rat model of AD.

Correspondence to: Professor Min Chen, Medical Technology School, Qiqihar Medical University, 333 Bukui Street, Jianhua District, Qiqihar 161006, P.R. China
E-mail: liqiangchina@yeah.net

Key words: Alzheimer's disease, Morris water maze, free radical, β amyloid protein, donepezil hydrochloride

Introduction

Alzheimer's disease (AD) is a progressive degenerative disease of the central nervous system with clinical and pathological characteristics (1). In the brains of AD patients, amyloid deposits, whether in senile plaques or neurofibrillary tangles or in parts of the vascular walls of the brain, become β-amyloid proteins (Aβ) (2). A previous study has confirmed that mutation of the amyloid precursor protein (APP) gene may cause abnormalities in APP metabolism and Aβ deposition in the brain (3). APP gene mutation is found in 1 in 4 familial AD patients (4). Esh *et al* used a mutated APP gene (Val717-Phe717) to create a transgenic mouse model, which significantly expresses 717-mutated APP and gradually forms the neuropathological features of AD, including the appearance of β-amyloid plaques (5).

The superoxide radical produced by the metabolic process of aerobic cells has harmful effects, including biological damage and senility. The oxygen radical promotes the accumulation of Aβ and induces hyperactivity in the brain, causing neurodegeneration and, hence, AD (6). Previous studies have identified that the body's ability to scavenge free radicals decreases with increasing age. Free radicals accumulate in the body and damage tissues and cells to accelerate the aging process (7) and are counteracted through a series of antioxidases, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). When the normal defensive function declines, oxidative damage occurs and free radicals damage neurons and other cells (8).

Numerous studies have confirmed that AD accompanies various neurotransmitter disorders and that the acetylcholine (ACh) system is more affirmative in relation to AD (9). The central ACh neurotransmitter has important regulatory functions in learning and memory behavior. The maintenance of the normal functioning of this regulatory function is essential to ensure normal learning and memory (10). Furthermore, functional deficiency of the cholinergic system is closely related to AD, as confirmed by a number of autopsies and as accepted by most researchers. Numerous reports have shown that the expression of ACh and the metabolic enzyme, choline acetyltransferase (ChAT), is significantly decreased in the cerebral cortex and hippocampus in AD patients. The numbers of N receptors in the cerebral cortex and M receptors

in the neuron-damaged area are also decreased, implying that AD is related to the loss of neurons in the nucleus basalis of Meynert (11). Certain studies have revealed that the degree of ACh decrease positively correlates with the severity of dementia (12).

Materials and methods

Animals and grouping. A total of 80 healthy male Sprague-Dawley (SD) rats were purchased from Beijing Weitong Lihua Test Animal Technology Co., Ltd (Beijing, China). Their weights were 280±10 g. They were randomly divided into four groups, namely, the blank, sham, model and donepezil hydrochloride (HCl) treatment groups. This study was approved by the Animal Care and Use Committee of Qiqihar Medical University.

Preparation of animal models (13). Following narcotization with 1% pentobarbital sodium (40 mg/kg), the rats were fixed on a stereotaxic apparatus. The skins on their cranial bones were preserved and the skin around the surgery area was routinely disinfected. A 2-3 cm incision was made along the cranial midline and the periosteum was detached. Then, a '+'-shaped suture and a lambdoid suture 3.0 mm behind the bregma were exposed and two 2.2 mm side openings were made around the central line. The skull was drilled using a dental auger and a microscale injector was inserted at 2.8 mm for the 1 μl solution injection. The injection time was 5 min and the needle was retained for 5 min to guarantee the diffusion of the solution. The needle was then pulled out slowly and the opening created in the skull was blocked with dental mud. The skin was disinfected with penicillin powder and then sutured. The other side of the hippocampus was injected following the same method. The model and donepezil HCl groups were injected with 10 μg/μl $A\beta_{1\text{-}40}$. The sham group was injected with normal saline and no treatment was administered to the blank group. Finally, 0.33 mg/kg/day donepezil HCl was injected into the donepezil HCl group through the stomach. Normal saline of a similar volume to that of the donepezil HCl group was injected into the stomachs of the sham and model groups. The injections were administered once daily for four weeks.

Morris water maze test. The pool was emptied of clear water. Then, 2 kg of fresh milk powder was dissolved to form a solution and poured into the pool to provide an opaque ivory color. The water temperature was maintained at 24-25˚C and the pool was placed at the center of the laboratory. Markers of different colors and shapes were placed in the four quadrants of the pool well. Reference objects around the pool were used as distant vision hints and were left unchanged throughout the Morris water maze test. During the test, the rats were placed into the water at any point of the other three quadrants facing the pool well. The test was conducted for five days, with one test conducted in every quadrant each morning for 1 min at every turn. The rats who could not find the platform were placed on the platform and allowed to stand for 15 sec. Those that found the platform were also allowed to stand on it for 15 sec. The training would then end. The training was conducted for four days. On the fifth day, the platform was removed and the rats were allowed to swim for 1 min. The 1-min swimming distance in the platform quadrant and other three quadrants was recorded and the percentage of the swim distance in the fourth platform quadrant (l_4) to the total swim distance (l total) was calculated.

Preparation of specimens. Following the behavioral testing, five rats from each group were sacrificed by decapitation and the brains were removed and quickly frozen in liquid nitrogen and then prepared for RNA extraction. A total of nine rats from each group were sacrificed by decapitation and the brains were removed and quickly frozen. The left brains were weighed and prepared as brain homogenates. The right brains of six rats were fixed in a timely manner using 4% paraformaldehyde solution for light microscopic observation (immunohistochemistry).

Determination of GSH-Px and CAT. The determination of the GSH-Px, CAT and protein contents of the brain homogenates was performed according to the instructions of the GSH-Px and CAT assay kit (Jiancheng Bioengineering, Nanjing, China).

Immunohistochemistry. The brain tissue obtained was sliced according to the paraffin method to a thickness of 5 μm. The wax was removed from the slice to allow hydration and the tissues were incubated with 3% H_2O_2 for 5 min to remove the endogenous CAT activity. The first antibody (dilution ratio of 1:200) was introduced and remained in contact with the tissue overnight at 4˚C and the sample was then washed thrice with PBS liquid for 2 min. The second antibody (goat anti-rabbit) working solution was then added to the sample which was maintained at room temperature for 20 min. The sample was then washed thrice with PBS liquid for 2 min. The DAB coloration was observed under a light microscope. The sample was washed with distilled water to terminate the chromogenic reaction, redyed using hematoxylin for 2 min and gradually dehydrated with alcohol (80, 90, 95 and 100%) for 2 min for each alcohol concentration. The tissues were rendered transparent with dimethylbenzene and fixation was tested on a slide with resin.

RT-PCR. The total brain RNA was extracted using a TRIzol kit (Invitrogen, Carlsbad, CA, USA). A total of 100 mg rat brain tissue was placed into a glass homogenizer. The total RNA extraction was performed according to the kit's instructions provided by the manufacturer. First strand cDNA synthesis was performed using Superscript II reverse transcriptase (Invitrogen, Shanghai, China). The reverse transcription reaction was performed according to the kit's instructions and synthetic cDNA was used to create the polymerase chain reaction. The primer sequences were as follows: the forward and reverse sequences for APP were 5'-TGGGTTGACAAACATCAAGACAGAA-3' and 5'-GCACCTTTGTTTGAACCCACATC-3', respectively, with 135 bp produced. Those of the β-secreted enzyme 1 (BACE1) were 5'-TGGTGGACACGGGCAGTAGTAA-3' and 5'-TCGGAGGTCTCGGTATGTACTGG-3', respectively, with 104 bp produced. Finally, those for phosphoglyceraldehyde dehydrogenase (GAPDH) were 5'-GACAACTTTGGCATC GTGGA-3' and 5'-ATGCAGGGATGATGTTCTGG-3',

Table I. Learning and memory ability of AD rats and the effects of donepezil hydrochloride on them (mean ± SD).

Groups	No.	Swim distance in each quadrant (cm)				
		l_1	l_2	l_3	l_4	l_4/ltotal(%)
Blank	20	323.89±40.58	391.35±38.56	334.90±49.38	758.01±72.14[b]	41.95±3.65
Sham	19	312.12±48.91	402.52±42.35	339.88±53.47	750.99±70.11[b]	41.64±3.27
Model	18	346.57±50.65	411.18±44.17	443.31±48.70	389.78±58.34	24.44±2.76[c]
Donepezil HCl	19	317.99±37.45	396.44±49.54	501.11±44.39	721.01±45.20[a]	37.28±2.42[d]

AD, Alzheimer's disease; HCl, hydrochloride. Compared with other quadrants [a]P<0.05, [b]P<0.01. [c]Compared with the sham groups, P<0.01. [d]Compared with the model group, P<0.01.

Table II. Levels of GSH-Px and CAT in AD rat brain tissue and the effects of donepezil hydrochloride on them (mean ±SD).

Groups	No.	CAT (U/mg x protein)	GSH-Px (U/mg x protein)
Blank	9	17.58±1.59	173.59±16.26
Sham	9	16.55±1.29	164.60±14.70
Model	9	11.05±1.49[a]	123.34±14.02[a]
Donepezil HCl	9	13.10±1.40[c]	141.44±13.12[b]

GSH-Px, glutathione peroxidase; CAT, catalase; AD, Alzheimer's disease; HCl, hydrochloride. [a]Compared with the blank and sham groups, P<0.01; [b]Compared with the model group P<0.01; [c]Compared with the sham groups, P<0.01.

Table III. Levels of AChE and ChAT in AD rat brain and the effects of donepezil hydrochloride on them (mean ± SD).

Groups	No.	AChE	ChAT
Blank	6	0.086±0.011	0.115±0.024
Sham	6	0.079±0.010	0.094±0.026
Model	6	0.045±0.008[a]	0.046±0.012[a]
Donepezil HCl	6	0.065±0.014[c]	0.072±0.014[b]

AChE, acetylcholinesterase; ChAT, choline acetyltransferase; AD, Alzheimer's disease; HCl, hydrochloride. [a]Compared with the blank and sham groups, P<0.01; [b]Compared with the model group P<0.01; [c]Compared with the sham groups, P<0.01.

respectively. The annealing temperatures of APP, BACE1 and GAPDH were 56, 54 and 55˚C, respectively.

The PCR product (5 μl) was examined via agarose gel electrophoresis. The results were observed and recorded in an ultraviolet box. Image acquisition and analysis of the strength of the electrophoresis bands of the PCR product were carried out using an image acquisition and analysis system.

Statistical analysis. The data are presented as the means ± SD and the statistical analysis was performed using the SPSS 11.0 software. The LSD method was used in the variance analysis and pair comparisons in multiple groups.

Results

Effects of donepezil HCl on learning and memory ability in a rat model of AD. As shown in Table I, the swim distance of the fourth quadrant was significantly different (P<0.05 or <0.01) from those of the other quadrants in the blank, sham and donepezil HCl groups. No significant differences were identified in the swim distance for each quadrant in the model group. These results indicate that the rat memories of the platform quadrant in the blank, sham and donepezil HCl groups were improved compared with those of the model group rats. The ratios of the swim distance in the fourth platform quadrant (l_4) to the total swim distance (l total) of each group (l_4/l total) were calculated. The l_4/l total of the model group was significantly lower than that of the blank and sham groups (P<0.01). However, the l_4/l total ratio of the donepezil HCl group was significantly increased compared with that of the model group (P<0.01).

GSH-Px and CAT levels. As shown in Table II, no significant differences were identified between the GSH-Px and CAT levels in the rats of the blank and sham groups (P>0.05). The GSH-Px and CAT levels in the rat brain tissues significantly decreased following AD modeling compared with those of the blank and sham groups (P<0.01). Moreover, the GSH-Px and CAT activities in the AD rat brains significantly increased following donepezil HCl treatment compared with those in the rat brains of the model group (P<0.05 or <0.01).

Acetylcholinesterase (AChE) and ChAT expression. As shown in Table III, no significant differences were found between the AChE and ChAT levels in the rats of the blank and sham groups (P>0.05). Moreover, the AChE and the ChAT expression decreased following AD modeling and the differences between the AChE and ChAT expression levels in the rats of the model group and those of the blank and sham groups rats were significantly different (P<0.01). The AChE and ChAT expression in the brain tissue was increased following donepezil HCl treatment (P<0.05 or <0.01). These results correspond with the known functional mechanism of cholinesterase inhibitor reagents.

Table IV. mRNA expression of APP in AD rat brain tissue and the effect of donepezil hydrochloride on it (mean ± SD).

Groups	No.	Mean ± SD
Blank	5	0.82±0.21
Sham	5	0.92±0.21
Model	5	1.74±0.23[a]
Donepezil HCl	5	1.14±0.19[b]

APP, amyloid precursor protein; AD, Alzheimer's disease; HCl, hydrochloride. [a]Compared with the blank and sham groups, P<0.01. [b]Compared with the model group, P<0.01.

Table V. mRNA expression of BACE1 in AD rat brain tissue and the effect of donepezil hydrochloride on it (mean ± SD).

Groups	No.	Mean ± SD
Blank	5	0.54±0.14
Sham	5	0.61±0.11
Model	5	1.12±0.34[a]
Donepezil HCl	5	0.74±0.17[b]

BACE1, β-secreted enzyme 1; AD, Alzheimer's disease. [a]Compared with the blank and sham groups, P<0.01. [b]Compared with the model group, P<0.05.

APP and BACE1 mRNA expression. The results in Table IV show no significant difference between APP levels in the rats of the blank and sham groups (P>0.05). The APP mRNA expression levels in rats of the model group were significantly increased compared with those in rats of the blank and sham groups (P<0.01), whereas those in the donepezil HCl group rats were significantly decreased compared with those in the model group rats (P<0.01).

Moreover, the results in Table V show no significant difference between the BACE1 levels in the rats of the blank and sham groups (P>0.05). The BACE1 mRNA expression levels in the rats of the model group were significantly increased compared with those in the rats of the blank and sham groups (P<0.01; Table V), whereas those of the donepezil HCl group rats were significantly decreased compared with those of the model group rats (P<0.05).

Discussion

The clinical manifestations of AD include hypophrenia, recent memory loss and obstacles in the related capacity for action (15). Thus, learning and memory are important factors in AD diagnosis and for the evaluation of its treatment (16). In the present experimental study, the Morris water maze system was used to examine the learning and memory abilities of each group of rats. The swim trajectories of the normal and approximately normal rats were predominantly scattered in the fourth platform quadrant. The memory-deficient rats aimlessly searched for a means of escape, and thus, their swim trajectories were chaotic. Moreover, the distance of aimless swim significantly increased as the spatial location memory of the rats decreased and they could not remember the location of the platform. The results showed that the swim distance in the fourth quadrant for the model group was significantly different from that in the other quadrants for the blank, sham and donepezil HCl groups (P<0.05 or <0.01).

The increased production of free radicals in AD patients is one of the causes of the changes in brain structure and function (17). Studies have shown that free radicals may promote the accumulation of Aβ and induce hyperactivity in the brain, causing neurodegeneration and, hence, AD. The increase in oxygen radicals may result in tau protein abnormal phosphorylation and neurofibrillary tangles, therefore causing functional degradation of the nerve cell, or even death, and aggravating the course of AD (18). Other studies have shown that free radicals may induce cell apoptosis and cause AD. The defensive reaction of cells against free radicals is of two types. The first is the defensive reaction of enzymes, including SOD, CAT and GSH-Px. SOD converts superoxide free radicals into H_2O_2. The low concentration of H_2O_2 is converted into water and molecular oxygen. GSH is used as proton donor and the high concentration of H_2O_2 is removed by CAT (19). CAT and GSH-Px are significant enzymes in the removal of free radicals as they transform the H_2O_2 produced by SOD, catalyzing the disproportionation of two superoxide radicals into water (20). The present experiment shows that the AD modeling by bilateral $A\beta_{1\text{-}40}$ injection into the hippocampus of rats may inhibit the free radical defensive function and lead to oxidative damage. Donepezil HCl significantly increases the levels of GSH-Px and CAT. The antioxidase activities of CAT or GSH-Px may be adjusted for indirect antioxidation.

ACh participates in the regulation of the neuronal activity of the hippocampus and neocortex, it is widely spread along the synaptic cleft of the cholinergic synapse and is neurotransmitted for the promotion of learning and memory. ACh is also an important index that reflects cholinergic nerve function and is significant in the memory and cognition damage of AD patients (21). ACh is synthesized by the ChAT-catalyzed reaction of choline with acetoacetyl-CoA and resolved by AChE. ChAT and AChE together maintain the dynamic balance of ACh (22). AChE has high catalytic activity and inactivates ACh by resolving it into choline and acetic acid to ensure effective transfer at the cholinergic nerve. The activity of AChE directly reflects the functional state of the cholinergic system.

Cholinesterase inhibitors have been used clinically to treat AD. Inhibiting the activity of cholinesterase may increase the ACh content of the brain and lead to a recovery of cholinergic nerve transmission. Cholinesterase inhibitors have achieved beneficial treatment effects in mild and moderate AD patients. ChAT is the rate-limiting enzyme of ACh synthesis, a focus of studies of cholinergic neurons and an indirect evaluation index for the levels of ACh that are released. A number of studies have shown decreases in the activity of ChAT of 49-90% in the cerebral cortex, hippocampus and basal nucleus of telencephalon group of AD patients. Moreover, the degree of the decrease in activity is closely related to the degree of dementia (23). ACh is very unstable and difficult to determine due to its rapid rate of hydrolysis. Therefore, the functioning

of the cholinergic system is usually observed through the activities of AChE and ChAT. The present experimental results show that in the AD model group of rats the AChE expression increased and the ChAT expression decreased.

Aβ production is the initial step in SP formation and very high concentrations of Aβ may be identified in brains of AD patients. Aβ is toxic to neurons and causes the denaturation and death of neuronal cells (24). Moreover, Aβ may worsen the damaging effects of free radicals, increase the inflammatory response to cell factor, disturb ionic equilibrium and induce cell apoptosis (25). The Aβ pathway is common to AD of various causes and is central to the pathological mechanism of AD. Aβ has been confirmed to be generated from the gene that codes APP (26). Jeong *et al* found that the mRNA levels of β-APP and APP in the brain hippocampus increased with age when they tested senile aged mice (SAM). Moreover, they stated that the excessive expression of hippocampal APP was related to the memory loss of the SAM (27). Therefore, the decrease in the APP mRNA expression may inhibit Aβ accumulation in the brain. The APP protein processing paths have predominantly amyloid and non-amyloid peptide sources. β and γ secretases together induce APP to produce the pathological Aβ (28). β-secretase is a pivotal rate-limiting enzyme of the Aβ production process. The Aβ level may be regulated by the activation or suppression of β-secretase. Therefore, the use of β-secretase, also called BACE or BACE1, as a target is expected to become an ideal method of AD treatment (29).

Acknowledgements

This research was supported by the Research Foundation of Education Bureau of Heilongjiang Province, China (Grant no. 11541413 and 12521619).

References

1. Lee DY, Fletcher E, Martinez O, *et al*: Vascular and degenerative processes differentially affect regional interhemispheric connections in normal aging, mild cognitive impairment, and Alzheimer disease. Stroke 41: 1791-1797, 2010.
2. Aluise CD, Robinson RA, Beckett TL, *et al*: Preclinical Alzheimer disease: brain oxidative stress, Abeta peptide and proteomics. Neurobiol Dis 39: 221-228, 2010.
3. Zhou ZD, Chan CH, Ma QH, Xu XH, Xiao ZC and Tan EK: The roles of amyloid precursor protein (APP) in neurogenesis: implications to pathogenesis and therapy of Alzheimer disease. Cell Adh Migr 5: 280-292, 2011.
4. Armstrong J, Boada M, Rey MJ, Vidal N and Ferrer I: Familial Alzheimer disease associated with A713T mutation in APP. Neurosci Lett 370: 241-243, 2004.
5. Esh C, Patton L, Kalback W, *et al*: Altered APP processing in PDAPP (Val717→Phe) transgenic mice yields extended-length Abeta peptides. Biochemistry 44: 13807-13819, 2005.
6. Ceballos-Picot I, Nicole A and Sinet PM: Cellular clones and transgenic mice overexpressing copper-zinc superoxide dismutase: models for the study of free radical metabolism and aging. EXS 62: 89-98, 1992.
7. Moon EY, Oh JM, Kim YH, Ryoo IJ and Yoo ID: Clitocybins, novel isoindolinone free radical scavengers, from mushroom *Clitocybe aurantiaca* inhibit apoptotic cell death and cellular senescence. Biol Pharm Bull 32: 1689-1694, 2009.
8. Percy ME, Dalton AJ, Markovic VD, *et al*: Red cell superoxide dismutase, glutathione peroxidase and catalase in Down syndrome patients with and without manifestations of Alzheimer disease. Am J Med Genet 35: 459-467, 1990.
9. Loewenstein DA, Acevedo A, Czaja SJ and Duara R: Cognitive rehabilitation of mildly impaired Alzheimer disease patients on cholinesterase inhibitors. Am J Geriatr Psychiatry 12: 395-402, 2004.
10. Lamirault L, Guillou C, Thal C and Simon H: (-)-9-Dehydrogalanthaminium bromide, a new cholinesterase inhibitor, enhances place and object recognition memory in young and old rats. Neurobiol Learn Mem 80: 113-122, 2003.
11. Gibb WR, Mountjoy CQ, Mann DM and Lees AJ: Pathological study of the association between Lewy body disease and Alzheimer's disease. J Neurol Neurosurg Psychiatry 52: 701-708, 1989.
12. Tohgi H, Abe T, Hashiguchi K, Saheki M and Takahashi S: Remarkable reduction in acetylcholine concentration in the cerebrospinal fluid from patients with Alzheimer type dementia. Neurosci Lett 177: 139-142, 1994.
13. Li J, Wang G, Liu J, *et al*: Puerarin attenuates amyloid-beta-induced cognitive impairment through suppression of apoptosis in rat hippocampus in vivo. Eur J Pharmacol 649: 195-201, 2010.
14. Kim JW, Lee DY, Choo IH, *et al*: Microstructural alteration of the anterior cingulum is associated with apathy in Alzheimer disease. Am J Geriatr Psychiatry 19: 644-653, 2011.
15. Saber AJ and Cain DP: Combined beta-adrenergic and cholinergic antagonism produces behavioral and cognitive impairments in the water maze: implications for Alzheimer disease and pharmacotherapy with beta-adrenergic antagonists. Neuropsychopharmacology 28: 1247-1256, 2003.
16. Wang L, Khan A, Csernansky JG, *et al*: Fully-automated, multi-stage hippocampus mapping in very mild Alzheimer disease. Hippocampus 19: 541-548, 2009.
17. Polidori MC and Mecocci P: Plasma susceptibility to free radical-induced antioxidant consumption and lipid peroxidation is increased in very old subjects with Alzheimer disease. J Alzheimers Dis 4: 517-522, 2002.
18. Moreira PI, Sayre LM, Zhu X, Nunomura A, Smith MA and Perry G: Detection and localization of markers of oxidative stress by in situ methods: application in the study of Alzheimer disease. Methods Mol Biol 610: 419-434, 2010:
19. Aksenov MY, Tucker HM, Nair P, *et al*: The expression of key oxidative stress-handling genes in different brain regions in Alzheimer's disease. J Mol Neurosci 11: 151-164, 1998.
20. Evereklioglu C, Er H, Doganay S, *et al*: Nitric oxide and lipid peroxidation are increased and associated with decreased antioxidant enzyme activities in patients with age-related macular degeneration. Doc Ophthalmol 106: 129-136, 2003.
21. Bales KR, Tzavara ET, Wu S, *et al*: Cholinergic dysfunction in a mouse model of Alzheimer disease is reversed by an anti-A beta antibody. J Clin Invest 116: 825-832, 2006.
22. Xiao F, Li XG, Zhang XY, *et al*: Combined administration of D-galactose and aluminium induces Alzheimer-like lesions in brain. Neurosci Bull 27: 143-155, 2011.
23. Lestaevel P, Bensoussan H, Racine R, Airault F, Gourmelon P and Souidi M: Transcriptomic effects of depleted uranium on acetylcholine and cholesterol metabolisms in Alzheimer's disease model. C R Biol 334: 85-90, 2011.
24. Sultana R, Robinson RA, Di Domenico F, *et al*: Proteomic identification of specifically carbonylated brain proteins in APP(NLh)/APP(NLh) x PS-1(P264L)/PS-1(P264L) human double mutant knock-in mice model of Alzheimer disease as a function of age. J Proteomics 74: 2430-2440, 2011.
25. Monji A, Utsumi H, Ueda T, *et al*: Amyloid-beta-protein (A beta) (25-35)-associated free radical generation is strongly influenced by the aggregational state of the peptides. Life Sci 70: 833-841, 2002.
26. Tamaoka A, Fraser PE, Ishii K, *et al*: Amyloid-beta-protein isoforms in brain of subjects with PS1-linked, beta APP-linked and sporadic Alzheimer disease. Brain Res Mol Brain Res 56: 178-185, 1998.
27. Jeong SJ, Kim K and Suh YH: Age-related changes in the expression of Alzheimer's beta APP in the brain of senescence accelerated mouse (SAM)-P/10. Neuroreport 8: 1733-1737, 1997.
28. Mishra S and Caflisch A: Dynamics in the active site of β-secretase: a network analysis of atomistic simulations. Biochemistry 50: 9328-9339, 2011.
29. Singer O, Marr RA, Rockenstein E, *et al*: Targeting BACE1 with siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model. Nat Neurosci 8: 1343-1349, 2005.