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RESEARCH ARTICLE

Evaluation of the antioxidant activity of donepezil - *in vitro* study

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ABSTRACT

Background: Donepezil is a cerebroselective anticholinesterase which is majorly used for memory enhancement in Alzheimer's disease (AD). The main action is through increasing central cholinergic neurotransmission but also indicated that it protects neurons from neuroinflammation mediated by free radicals which has been implicated in the pathology of the AD. Hence, in this study, we have evaluated the antioxidant potential of drug using 1,1 diphenyl 2 picrylhydrazine (DPPH) and nitric oxide (NO) synthase assay. It was found that donepezil has significant antioxidant activity which can be useful as antioxidant also along with increasing central cholinergic neurotransmission. **Objective:** The objective of the study was to evaluate *in vitro* antioxidant property of donepezil. **Materials and Methods:** In this study, we have demonstrated *in vitro* antioxidant activity of donepezil. DPPH and NO synthase assay tests were done for different concentrations of donepezil. **Results:** In our study, it showed that the free radical scavenging activity of donepezil was less in lower concentration and increased in the higher concentration in DPPH assay. The free radical scavenging activity of drug donepezil was 33.5% at 10 µg/ml and 42.3% for the concentrations of 1000 µg/ml in DPPH assay. NO scavenging activity was 1% at 10 µg/ml and 14.9% at 1000 µg/ml. **Conclusion:** Thus, the *in vitro* antioxidant analysis of donepezil was proved to be a potent antioxidant.

KEY WORDS: Alzheimer's Disease; 1,1 diphenyl 2 picrylhydrazine; Nitric Oxide; Free Radicals; Donepezil

INTRODUCTION

Alzheimer's disease (AD) is a chronic degenerative disease of central neurons and brain cells, primarily in hippocampus and cortex.[1] The risk of AD rises exponentially with age and varies from 12% to 19% for women and 6% to 10% for men over the age of 65 years.[2] It has been shown that up to 47% of individuals above the age of 80 years develop AD.[3] AD patients live on an average about 8 years after initial diagnosis even though it can last for 20 years. It is manifested as impairment of thought, memory, and language abilities. Several varied proposed theories of AD etiology are amyloid beta toxicity,[4] tauopathy,[5] inflammation,[6,7] and oxidative stress.[8-13] Free radical damage and oxidative stress play a major role in the pathogenesis of AD. Oxidative stress is a potential source of damage to proteins, lipids, sugars, and deoxyribonucleic acid within cells. Any imbalance between the intracellular production of free radicals or reactive oxygen species and antioxidant defense mechanism leads to oxidative stress.[8,11,12] Oxidative stress is one of the major factor implicated in degeneration of cholinergic neurons in AD.

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At present, the Vitamin E is used as promising antioxidant therapy in moderately severe AD.[14,15] Most of the currently known antioxidants have limited ability to cross blood-brain barrier. Therefore, the development of smaller molecules which are readily/easily crosses the barrier into the brain offers much promise. At present, cerebroselective anticholinesterase drugs such as donepezil, rivastigmine, and galantamine are

mainstay therapy in AD. Donepezil is the major drug used in the treatment of AD for an increase in the cerebral cholinergic neurotransmission. Donepezil is reversible cerebroselective anticholinesterase produces measurable improvement in several cognitive and noncognitive scores in the AD. The benefit is ascribed to an elevation of Ach level in the cortex, especially in the surviving neurones that project from basal forebrain to cerebral cortex and hippocampus. It is administered only once at bedtime due to its long half-life (70 h) and its cholinergic side effects are mild/minimal.[16] Moreover, it can also be used in relatively severe cases of the AD. The drug molecule with multiple actions such as increasing Ach levels and antioxidant will be helpful in the AD rather than using multiple medications with individual actions. The ability of cholinesterase inhibitors acting as antioxidants is an important aspect for neuroprotection. However, this aspect has not been properly investigated. Therefore, the present study was aimed at *in vitro* antioxidant effect of donepezil by 1,1 diphenyl 2 picrylhydrazine (DPPH) and NO assay method.

MATERIALS AND METHODS

Free radical scavenging activity of donepezil was determined by DPPH assay method as per Yohozowa *et al*. 1998.[17] The nitric oxide (NO) radical scavenging activity of donepezil was done using the method of Alderson *et al*. in 2001.[18]

DPPH Scavenging Activity

The free radical scavenging activity of donepezil was determined using DPPH radical scavenging activity using the method of Yohozowa *et al*.[17] This assay is based on the principle of reduction of the absorbance of ethanol solution of DPPH by a free radical scavenger. Reagents required for this assay were DPPH and ethanol. The reaction mixture containing 1 ml of DPPH solution (200 µm in ethanol) with different concentration of the test drug (10, 50, 100, 200, 400, 800, 1000 µg/ml) was shaken and incubated in the dark for 20 min at room temperature. The resultant absorbance was recorded at 517 nm using a spectrophotometer. Donepezil free radical scavenging activity (the percentage inhibition) was calculated using the formula:

$$\text{Percentage inhibition}=(Abs_{(control)}-Abs_{(Sample)})/Abs_{(control)}\times 100$$

NO Scavenging Activity

The NO radical scavenging activity was done using the method of Alderson *et al*.[18] It is based on the principal of inhibition of NO radical (which is generated from sodium nitroprusside in phosphate buffered saline with the addition of Griess reagent). Griess reagent contains 1% sulfanilamide, 2% phosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride in 100 ml of distilled water. 3 ml of a reaction mixture containing sodium nitroprusside (10 mM in phosphate buffer saline) and various concentrations (10, 50, 100, 200, 400, and 800 µg/ml) of the test drug were incubated at 37°C for 4 h. To the incubation solution, 0.5 ml of Griess reagent was added, and the absorbance was read at 546 nm using a spectrophotometer. The free radical scavenging activity of donepezil (the percentage inhibition) was calculated using the formula:

$$\text{Percentage inhibition}=(Abs_{(control)}-Abs_{(Sample)})/Abs_{(control)}\times 100$$

RESULTS

The NO synthase assay was done for different concentrations of Vitamin C and donepezil from 10 µg/ml to 1000 µg/ml. The percentage inhibition of Vitamin C was 56%, 64%, 74%, 79%, 83%, 87%, and 92%, and the drug donepezil was 1%, 2%, 5%, 6.5%, 9.7%, 11.6%, and 14.9% for the concentration of 10 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, 800 µg/ml, and 1000 µg/ml. The NO radical scavenging activity of donepezil showed dose-dependent gradual increase in free radical activity. The percentage inhibition was 1% at 10 µg/ml and 14.9% at 1000 µg/ml concentrations [Table 1].

DPPH assay was done for different concentrations of Vitamin C and donepezil from 10 µg/ml to 1000 µg/ml. The percentage inhibition of Vitamin C 91%, 94%, 94%, 95%, 95.5%, 94%, and 94%, and for drug donepezil was 33%, 37%, 38%, 39%, 41%, 41%, and 42% for the concentrations of 10 g/ml, 50 g/ml 100 µg/ml, 200 µg/ml, 400 µg/ml, 800 µg/ml, and 1000 µg/ml, respectively [Table 2]. It showed dose-dependent gradual increase in free radical activity.

Table 1: Nitric oxide scavenging activity of donepezil

Concentration (µg/ml)	% Inhibition	
	Vitamin C	Donepezil
10	56.45±0.92	1.09±0.97
50	64.27±0.61	1.97±0.72
100	74.64±0.73	5.14±0.66
200	79.32±0.92	6.56±0.93
400	83.14±1.02	09.72±0.32
800	87.42±0.04	11.68±0.51
1000	92.12±0.52	14.97±0.84

Values are expressed as mean±SD of three experiments.
SD: Standard deviation

Table 2: DPPH scavenging activity of donepezil

Concentration (µg/ml)	% Inhibition	
	Vitamin C	Donepezil
10	91.02±0.02	33.50±0.33
50	94.59±0.03	37.44±0.02
100	94.96±0.11	38.45±0.01
200	95.14±0.08	39.60±0.01
400	95.51±0.06	40.97±0.11
800	94.78±0.04	41.70±0.13
1000	94.41±0.07	42.35±0.10

Values are expressed as mean±SD of three experiments.
SD: Standard deviation, DPPH: 1,1 diphenyl 2 picrylhydrazine

DISCUSSION

Free radicals produce oxidative stress. Most commonly involved free radicals are superoxide, hydroxyl, alkoxy, peroxy, and NO. Free radicals have extremely short half-lives ranging from nanoseconds to seconds. The hydroxyl radical is shortest, and NO is longest having half-life of one nanosecond and 1–10 s, respectively.[19] It is very well established that the involvement of oxidative damage of cellular molecules in neurodegenerative disorders. Oxidative damage also plays important role in the direct initiation of neurodegeneration also along with acting as by product or end product of neurodegenerative processes.

From our study, the free radical scavenging property as measured by NO synthase and DPPH method showed that percentage of inhibition increases with increase in the concentration of donepezil. It is an effective antioxidant with radical scavenging potency almost similar to Vitamin C. Hence, donepezil could also play a role of an antioxidant in AD. Further studies are required for a demonstration for antioxidant effect *in vivo*.

CONCLUSION

The study concluded that the antioxidant effect of donepezil is an additional mechanism that has been contributed to the treatment of Alzheimer's disease. From our study, it proves that donepezil has antioxidant property and it is dose dependent.

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