

Study of effects of donepezil and aspirin on working memory in rats using electroconvulsive shock model

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ABSTRACT

Background: Memory is the most common cognitive ability lost with dementia commonly seen in Alzheimer's disease (AD). Donepezil was the first cholinesterase inhibitor to be licensed in UK for AD. There is preliminary evidence that aspirin decreases the risk and delays the onset of AD. Low dose aspirin users had numerically lower prevalence of Alzheimer's dementia and had better cognitive function than non-users.

Methods: Retention of conditioned avoidance response (CAR) was assessed by using repeated electroconvulsive shocks (ECS) in rats. Rats were divided into five groups: control (pretreated with distilled water), ECS (150 V, 50 Hz, with intensity of 210 mA for 0.5 sec) pretreated, combined aspirin (6.75 mg/kg) and pretreated ECS, combined donepezil (0.32 mg/kg) and pretreated ECS, combined aspirin, donepezil and pretreated ECS groups. Data were analyzed using the Chi-square test and ANOVA.

Results: Findings show that administration of ECS daily for 8 days results in transient amnesia and disruption of retention of CAR. Aspirin and donepezil administration significantly increased the retention of CAR in comparison to ECS. However, aspirin failed to show an increase in the retention of CAR as compared to donepezil. The combination of the two drugs showed statistically significant increase in the retention of CAR than either of these drugs given alone.

Conclusion: Neuroinflammation plays an important role in the pathophysiology of neurodegenerative disorder like AD. Combination of aspirin with donepezil increased the nootropic and neuroprotective effect of aspirin and thus may hold great clinical significance in such disorders.

Keywords: Aspirin, Donepezil, Electroconvulsive shocks, Working memory

INTRODUCTION

Memory is a complex function of the brain that uses several storage buffers of capacity and duration. Working memory is the ability to maintain or hold temporary active representations of information for further processing or recall. The process is thought to have two components, short-term storage and executive processes that operate on the stored material. The memory fields may be modulated by many neurochemical systems, including dopamine, serotonin, noradrenaline, acetylcholine (ACh), gamma-amino butyric acid, and glutamate in highly differentiated ways, suggesting that modulation of these neurochemical systems may affect the different stages of working memory. Memory is the most common cognitive ability lost with dementia commonly seen in Alzheimer's disease (AD).

It is well-known that ACh is one of the important modulators of cognitive processes.¹ Donepezil was the first cholinesterase (ChE) inhibitor to be licensed in UK for AD. ChE inhibitors (Is) produce small improvements in cognitive and global assessments in AD. Acetylcholinesterase (AChE)/ChE-Is and memantine are licensed for symptomatic treatment of mild-moderate and moderate-severe forms of AD, respectively. High doses of the AChE-I donepezil were licensed in the USA for moderate-severe AD, and the association AChE/ChE-Is plus memantine was proposed for AD at this stage.² Its efficacy in severe dementia has been controversial.³

Aspirin, besides being an old anti-inflammatory drug, has been in use in other conditions like AD. There is preliminary evidence that aspirin decreases the risk and delays the

onset of AD. An inhibitory effect of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) on the processes contributing to AD and cognitive decline is plausible, though clinical evidence from clinical trials is lacking.⁴ Users of high dose aspirin had significantly lower prevalence of Alzheimer's dementia and had better cognitive function than non-users. Furthermore, low-dose aspirin users had numerically lower prevalence of Alzheimer's dementia and had better cognitive function than non-users.⁵

Behavioral models for studying memory shortage and recall and its manipulation by pharmacological agents do not represent the pathophysiology of AD. Thus, for induction and simulation of pathophysiology of underlying AD in experimental animals, amnesia is induced. Various agents used for the purpose are scopolamine, colchicine, etc. It has been found that after the completion of a course of electroconvulsive therapy (ECT), memory is impaired, and retrograde amnesia is seen in human beings. Amnesia can be induced in animals by chronic administration of electroconvulsive shocks (ECS) for 8-10 days, which act by facilitating serotonergic transmission.⁴ In the present study, ECS was given to rats in the ways closely mimicking the administration of ECT.⁴ Aspirin was compared with donepezil for the neuroprotective effects. The effect of a combination of aspirin and donepezil on the working memory was also assessed in rats.

METHODS

Animals

Experimentally naive Sprague-Dawley albino rats weighing between 150 and 200 g of either sex were used. The rats were maintained under standard conditions of temperature (25°C±5°C), relative humidity (55±10%) and a 12/12 hrs light/dark cycle. The rats were fed with commercially available rat pellet feed manufactured by Pranav Agro Food, Pune and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee.

Instruments, drugs and chemicals

Electro-convulsimeter and Cook's pole climbing apparatus were purchased from ST1 Instruments Pvt. Ltd. and New Neeta Manufacturers, Pune, India, respectively. Donepezil was purchased from Yashica Pharmaceuticals Pvt. Ltd., Mumbai, India. Aspirin was purchased from Ranbaxy Laboratories Ltd., Gurgaon, India.

Conditioned avoidance response (CAR)

This model was used to study the nootropic effects of aspirin and donepezil. The rats were trained for CAR by using Cook's pole climbing apparatus.⁶ The method of Fellow and Cook was used with some modifications. Each rat was allowed to acclimatize for 2 mins and was then exposed to

a buzzer noise. After 5 sec of putting on the buzzer, mild electric shocks were given through the stainless steel grid floor. The magnitude of the voltage was adequate (5-10 V) to stimulate the rat to escape from the floor and climb the pole. As soon as the rat climbed the pole, both the buzzer and the foot shocking were switched off. At least 10 such trials were given to each rat at an interval of 1 min/day for 10 days. After about 10 days training schedule, most of the rats learned to climb the pole within 5 sec of starting the buzzer, thus avoiding the electric foot shocks. Rats avoiding the foot shocks in all 10 out of 10 trials were considered to have developed CAR for further experiments.⁷

ECS induced disruption of memory

The rats were given a single maximal ECS for 0.5 sec duration through the crocodile clip ear electrodes from an electro-convulsimeter without any anesthesia. During the study, a single ECS was administered daily for 8 consecutive days.

Study drug administration

- i. Donepezil: donepezil was given in a dose of 0.32 mg/kg by intra-peritoneal route for 8 consecutive days in the animals after training for CAR
- ii. Aspirin: aspirin was given in a dose of 6.75 mg/kg by oral route for 8 consecutive days in the animals after training for CAR
- iii. ECS: ECS was given through electrodes applied to pinna daily for 8 consecutive days (150 V, 210 mA, for 0.5 sec)
- iv. Double distilled water was used as a vehicle for dissolving both the study drugs and was administered as a vehicle in the control group by oral route.

Grouping

The animals were divided into five different groups (n=10) as follows after training for CAR. They received the drugs by either intra-peritoneal route or oral route depending on the group.

C: Rats received distilled water for 8 days by oral route, which was used as a vehicle for study drugs and will serve as a control.

E: Rats received ECS daily for 8 days.

E + A: Rats received ECS and aspirin daily for 8 days.

E + D: Rats received ECS and donepezil daily for 8 days.

E + D + A: Rats received ECS, aspirin, and donepezil daily for 8 days.

On day 9, all rats were tested to see if they had retained the CAR. After 2 mins of the acclimatization period, each rat was exposed to the buzzer for 5 sec. Ten such trials were given at an interval of 1 min, without giving any foot shock. Rats responding by climbing the pole when exposed to the

buzzer noise were considered to have retained the CAR. Thus, retention of CAR in rats in each group was noted.

Statistical analysis

The result of the retention of CAR was analyzed using the Chi-square test. P<0.05 was considered to be statistically significant.

RESULTS

The percentage of rats showing retention of CAR was calculated in each group. The result is shown in Table 1. In control group, 50% of rats showed retention of CAR. This retention of CAR was significantly reduced in the rats treated with ECS (p<0.001).

The rats in Group E + A, Group E + D and Group E + D + A showed statistically significant increase in the retention of CAR as compared to Group E (p<0.0001). Rats in the Groups E + D and E + D + A showed statistically significant increase in the retention of CAR as compared to Group C. As compared to E + D + A, E + A and E + D showed statistically significant increase in the retention of CAR (p<0.01). When compared with control, increase in the retention of CAR in Group E + A was not statistically significant (p>0.05) (Figure 1).

DISCUSSION

AD is the most common form of dementia, affecting approximately 5% of the population over the age of 65 years. As the population ages, the social impact of AD

is becoming more critical. Thus, there is an urgent need for effective pharmacological treatments. ChE-Is have consistently shown symptomatic benefits and are now recognized as the standard treatments in the patients with mild-to-moderate AD.⁸ A non-competitive n-methyl-d-aspartate antagonist, memantine, is also available for the symptomatic treatment of moderately severe to severe patients. Unfortunately, neither class of drugs can halt or to slow the disease progression.

In the AD brain, degenerating neurons, deposits of aggregated Aβ and neurofibrillary tangles are the sites of inflammation. Amyloid plaques are associated with activated microglia and reactive astrocytosis. These cellular events are accompanied by an increased expression of members of the complement pathway (C1q, C3b, C3a, membrane attack complex), cytokines and chemokines (interleukin-1β, interleukin-6, tumor necrosis factor α and transforming growth factor β), and acute phase reactive proteins (α-2-macroglobulin and α1-antichymotrypsin) surrounding amyloid deposits, leading to inflammation. This pathophysiology could be the basis for the role of aspirin in delaying the onset of AD as the best-characterized action of NSAIDs is the inhibition of cyclooxygenase, leading to marked reduction in the biosynthesis of pro-inflammatory prostaglandins.⁹

The present study was planned to see if aspirin, when combined with donepezil, has additive effects on neuroprotection. Ghosh et al., have demonstrated nootropic and neuroprotective action of low-dose aspirin in rats.⁴ Recent laboratory findings also suggest a role of aspirin in neuroprotection.¹⁰ Daily low-dose aspirin treatment may reduce the global cognitive decline in older women at high risk for cardiovascular disease. The researchers found that women on regular low-dose acetylsalicylic acid (ASA) declined less on Mini-Mental State Examination at follow-up than those not on ASA. The same trend was seen with the other cognitive tests. The neuroprotective effect of aspirin is important in metabolic dysfunction. Aspirin seems to restore the neurotransmitter equilibrium in the cortex.⁷

Several epidemiological studies, but not all studies, have evidenced a reduced prevalence of AD among users of NSAIDs. The finding that increasing the duration of NSAID use is associated with a decreasing risk of AD, probably reflects the fact that the long-term users are taking NSAIDs at younger ages when the disease process is not yet started.¹¹

Our study shows that administration of single ECS daily for consecutive 8 days results in the disruption of retention of CAR. Aspirin and donepezil administration significantly increased the retention of CAR compared to control. Aspirin and donepezil also significantly prevented the attenuation of retention of CAR induced by ECS.

This study was designed to compare the effect of aspirin and donepezil on working memory in rats using ECS induced memory disruption and increase in the retention of CAR as

Table 1: Percentage of rats showing retention of CAR.

Groups	Retention of CAR%	
	Yes	No
C	50	50
E	20	80
E+A	60	40
E+D	70	30
E+D+A	80	20

CAR: Conditioned avoidance response

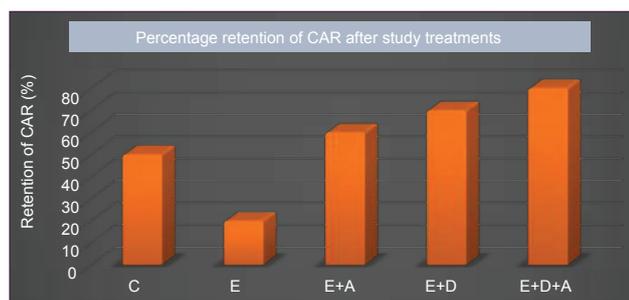


Figure 1: Percentage of rats showing the retention of conditioned avoidance response.

the comparative parameter. The results showed that aspirin and donepezil are equally effective in preventing the memory loss, and there is an additional benefit by combining the two drugs. Both these drugs have completely different profile regarding mechanism of action and hence the combination may be useful if given for a longer period of time and this warrants further highly specified animal experiments and also needs to be evaluated in humans. The additive effect of this combination, as shown in our study, may hold great clinical significance. Addition of a cost effective drug like aspirin not only lowers the overall cost of therapy, but it may also improve the memory retention in neurodegenerative disorders like AD.

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Conflict of interest: None declared

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