

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation and Comparison of Effects of Donepezil and Aspirin on Working Memory in Rats.

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ABSTRACT

To evaluate and compare the effects of donepezil and aspirin on working memory in rats. The rats were tested for retention of conditioned avoidance response (CAR) by using Cook's pole climbing apparatus. Rats were divided into 5 groups: control (distilled water), scopolamine (0.5 mg/kg), combined aspirin (6.75 mg/kg) and pre-treated scopolamine, combined donepezil (0.32 mg/kg) and pre-treated scopolamine, combined aspirin, donepezil and pre-treated scopolamine groups. The data was analyzed using Chi square test. Findings show that administration of single dose scopolamine 20 minutes before test run on the apparatus causes transient amnesia daily resulting in disruption of retention of CAR. Aspirin and donepezil significantly increased the retention of CAR in comparison to scopolamine. However aspirin failed to show increase in retention of CAR as compared to donepezil. But the combination of the two drugs showed statistically significant increase in the retention of CAR than either of these drugs given alone. Neuroinflammation plays an important role in the pathophysiology of neurodegenerative disorders like Alzheimer's disease and thus role of aspirin may hold great clinical significance.

Keywords: Working memory, aspirin, donepezil, conditioned avoidance response, scopolamine.

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INTRODUCTION

Alzheimer's disease was once thought to result from a cholinergic deficit alone; however researchers now believe that this view is simplistic. Studies using human neocortical tissues have shown that multiple neurotransmitters including dopamine, noradrenaline, serotonin and glutamate are decreased or dysregulated in AD [1-3]. Inflammatory process in cognitive brain centres may, at least in part, be responsible for causing the disease. Inflammation is the life saving mechanism that enables the body to build up a defensive attack against bacteria, parasites and viruses. Then, just as quickly, inflammation shuts down and healing begins, however, occasionally this process fails, inflammation becomes chronic and immunological defences mistakenly attack healthy cells, leading to permanent damage [4].

Aspirin, the old anti-inflammatory workhouse, is now approaching a status of a wonder drug as it is of benefit not only in inflammation but number of other conditions. There is preliminary evidence that aspirin reduces risk and retard the onset of AD [5].

Acetylcholinesterase inhibitors (AChEIs) are commonly used to boost the levels of acetylcholine (ACh), as an alternative to ACh agonist, to enhance the central cholinergic transmission, thereby improving cognitive function. Donepezil an acetylcholinesterase inhibitor has been in successful use for treatment of AD since few years. However, this compound is only approved for mild to moderate stages of AD, and confers only modest benefits in disease treatment [6].

Inflammatory process in cognitive brain centres may at least in part be responsible for causing dementia, the microglia being of central importance. The possibility that a well documented and non-expensive drug such as aspirin might serve to maintain cognitive function and reduce the development of Alzheimer's disease, makes it worth testing aspirin as an agent against cognitive decline. Users of high dose aspirin had significantly lower prevalence of Alzheimer's dementia and better cognitive function than non-users. Also, low dose aspirin users had numerically lower prevalence of Alzheimer's dementia and better cognitive function than non-users [5].

Cognitive impairment is seen in a wide variety of neurodegenerative disorders [7]. Working memory is a form of short-term memory with a limited capacity and an extremely rapid decay. Its impairment is more depictive of memory disorder in the Alzheimer's dementia [8].

Behavioural models for studying memory shortage and recall, and its manipulation by pharmacological agents, do not truly represent pathophysiology of AD. Thus, for the induction and stimulation of pathophysiology of underlying AD in experimental animals, amnesia is induced in animals. Various amnesia inducing agents are available, e. g., scopolamine, colchicines, etc. In the present study scopolamine was given to rats to evaluate and compare the effect of donepezil and aspirin on their working memory [9].

MATERIAL AND METHODS

Animals

Experimentally naive Sprague Dawley albino rats weighing between 150 - 200 g of either sex were used. The rats were maintained under standard conditions of temperature ($25^{\circ}\text{C} \pm 5^{\circ}\text{C}$), relative humidity ($55 \pm 10\%$) and a 12/12 hr 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 Ethical Committee.

Instruments, Drugs and chemicals

Cook's pole climbing apparatus was purchased from New Neeta Manufacturer, Pune, India. Donepezil was purchased from Yashica Pharmaceuticals Private limited, Mumbai, India. Aspirin was purchased from Ranbaxy Laboratories Ltd, Gurgaon, India. Scopolamine was obtained from Sigma Aldrich, St Louis, USA.

Conditioned Avoidance Response

This model was used to study the nootropic effects of aspirin and donepezil. The rats were trained for conditioned avoidance response by using Cook's pole climbing apparatus. The method of Fellow and Cook was used with some modifications. Each rat was allowed to acclimatize for two minutes and was then exposed to a buzzer noise. After 5 seconds of putting on the buzzer, mild electric shocks were given through the stainless steel grid floor. The magnitude of the voltage was adequate (5-10V) 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 per day for 10 days. After about 10 days training schedule, most of the rats learned to climb the pole within 5 seconds 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 conditioned avoidance response for further experiments [10].

Scopolamine induced disruption of memory

Rats trained for CAR received scopolamine (hydrobromide) in a dose of 0.5mg/kg by intraperitoneal route before administration of the study drugs. This is known to produce amnesia which will be used to evaluate the effect on learning and memory of the study drugs [11].

Study drug administration

- Donepezil: Donepezil was given in a dose of 0.32mg/kg by intraperitoneal route for eight consecutive days in the animals after training for conditioned avoidance response.
- Aspirin: Aspirin was given in a dose of 6.75mg/kg by oral route for eight consecutive days in the animals after training for conditioned avoidance response.

- Scopolamine: Scopolamine was dissolved in 0.9% saline solution and given in a dose of 0.5mg/kg by intraperitoneal route, 20 minutes prior to the test run on the apparatus.
- 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 5 different groups of 10 rats each after training for CAR. The animals received the drugs by either intra-peritoneal route or oral route depending on the group. The rats were divided into the following groups:

- **C (n = 10):** Rats received distilled water for 8 days by oral route and served as control.
- **S (n = 10):** Rats in this group received scopolamine 20 minutes prior to the test run on the apparatus for 8 days.
- **S + A (n = 10):** Rats in this group received scopolamine 20 minutes before administration of aspirin daily for 8 days.
- **S + D (n = 10):** Rats in this group received scopolamine 20 minutes before administration of donepezil daily for 8 days.
- **S + D + A (n = 10):** Rats in this group received scopolamine 20 minutes before administration of donepezil and aspirin daily for 8 days.

On day 9, all rats were tested to see if they had retained the conditioned avoidance response. After 2 min of acclimatization period, each rat was exposed to the buzzer for 5 seconds. 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 conditioned avoidance response. Retention of CAR in rats of each group was noted.

Statistical analysis

The result of the retention of CAR was analysed by the Chi-Square test. P value <0.05 was considered as significant.

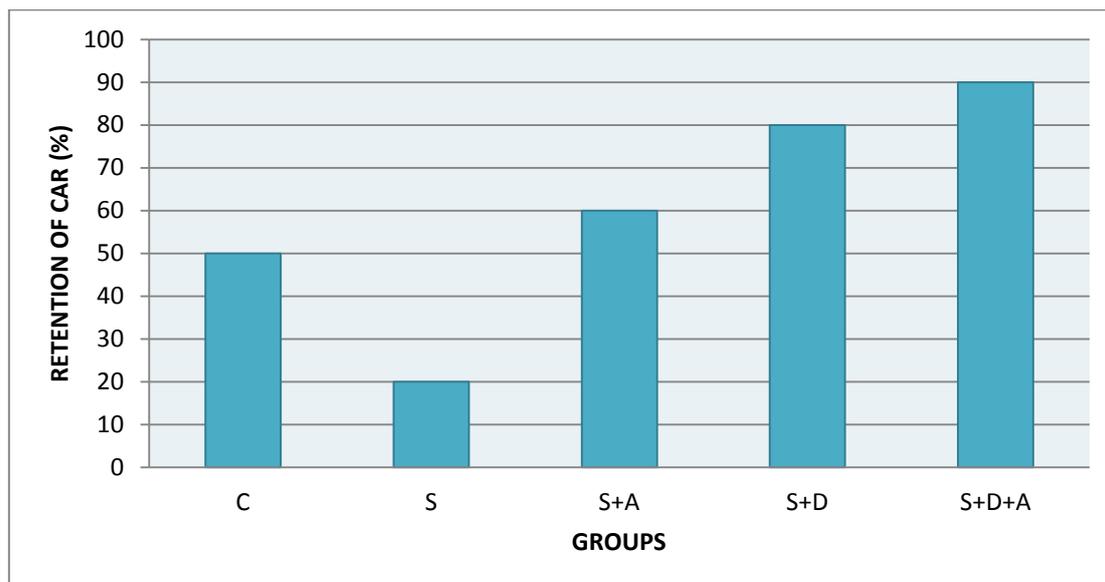
RESULTS

The percentage of rats showing retention of conditioned avoidance response was calculated in each group. The results are shown in Table 1. The rats in Group S+A, group S+D, group S+D+A showed statistically significant increase in retention of CAR as compared to group S ($p < 0.001$, highly significant difference is seen). Similarly, rats in group S+D, group S+D+A showed statistically significant increase in retention of CAR as compared to group C. But, when group S+A was compared with group C, the increase in the retention of CAR in group S+A was not statistically significant as compared to group C ($p > 0.05$, no significant difference is seen)(Figure 1).

Table 1: Percentage of rats showing retention of Conditioned Avoidance Response

Groups	Yes (Retention of CAR %)	No (Retention of CAR %)
C	50	50
S	20	80
S+A	60	40
S+D	80	20
S+D+A	90	10

Figure 1: Percentage of rats showing retention of Conditioned Avoidance Response



DISCUSSION

Alzheimer's disease is the most common form of dementia, affecting approximately 5% of the population over the age of 65. As the population ages, the social impact of AD is becoming more critical. Thus, there is an urgent need for effective pharmacological treatments. Cholinesterase inhibitors have consistently shown symptomatic benefits and are now recognized as the standard treatments in patients with mild-to-moderate AD. 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 is able to halt or slow the disease progression.

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

Several epidemiological studies, have evidenced a reduced prevalence of AD among users of NSAIDs. The finding that increasing 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 [12].

This study was designed to compare the effect of aspirin and donepezil on working memory in rats using scopolamine induced memory disruption and increase in the retention of CAR as the comparative parameter. Both drugs were well tolerated and there were no deaths during the study.

CONCLUSION

The results showed that aspirin and donepezil are equally effective in preventing memory loss and there is an additional benefit by combining the two drugs. Both these drugs have completely different profile regarding the mechanism of action, so 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 the therapy but it may also improve the memory retention in neurodegenerative disorders like Alzheimer's disease.

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