COMBINATION THERAPEUTIC INFLUENCE OF ASPIRIN AND RIVASTIGMINE ON SCOPOLAMINE INDUCED ALZHEIMER’S IN WISTAR RAT

Sudhakar Pachiappan*, Suchithra K. B., Suganeswari M. and Preetu Shukla K. M.

Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Tiruchengode-637 205, Tamil Nadu, India.

ABSTRACT

Objective: To investigate the combination therapeutic influence of aspirin and rivastigmine on scopolamine induced alzheimer’s in rats.

Materials and Methods: Twenty four male Albino Wistar Rats were enrolled in this study and are divided into 4 groups (six each). Memory impairment was induced by administration of scopolamine (1 mg/kg intraperitoneally). Group-I; receives normal saline, Group-II; receives scopolamine (1.0mg/kg/day i. p.), Group-III; Rivastigmine (0.3mg/kg/day p. o.) + Scopolamine (1.0 mg/kg day i. p.), Group-IV; Aspirin (6.75mg/kg/day p. o.) + Rivastigmine (0.3mg/kg/day p. o.) + Scopolamine (1.0 mg/kg/day i. p.) all the treatment carried out daily for 14 days. Cognitive and condition avoidance response was evaluated by using Morris Water Maze (MWM) and Pole climbing apparatus respectively. On 14th day brain was isolated for biochemical and Histopathological examination.

Results: In this study, Rivastigmine and aspirin combination showed that significant increase in cognitive and conditions avoid response. Brain histological investigation of scopolamine injected rats showed neurodegeneration, Aβ plagues and odema. This combination improves biochemical parameters as well as in the histological feature of the brain as compare to the rivastigmine alone treated group.

Conclusion: Aspirin and Rivastigmine combination possesses significant protective effect against neuroinflammation characterizing AD.

KEYWORDS: Scopolamine, alzheimer’s, neuroinflammation, rivastigmine, aspirin.
INTRODUCTION
Alzheimer's disease (AD) is a neurodegenerative and cause gradual memory loss. It is manifested by cognitive and behavioural derangements that markedly interfere with occupational and social functioning. This disease is characterized by loss of neurons and synapses in the cerebral cortex and certain sub cortical regions. Intra cellular accumulation of beta amyloid plaques (Aβ) and neurofibrillary tangles are the common features in brain afflicted by AD.\cite{1,2}

AD pathology is impaired cholinergic transmission, mitochondrial malfunction, neuronal stress oxidative damage, increased inflammatory mediators, synapse deprivation, deficiencies in steroid hormones and neuronal degeneration. Among these, it seems that cholinergic transmission impairment and neuroinflammation has a key role in development and progression of disease.\cite{3}

Neuroinflammation, as characterized by activation of glia (gliosis) and elevated presence of inflammatory molecules, is a common component of the normal aging brain, yet is exacerbated in AD. Numerous reports have indicated that neuroinflammatory process contributes to the pathogenesis of AD. Neuroinflammation plays an important role in the process of cerebral amyloid deposition, it has been shown that inflammatory cytokines such as Interleukin (IL)-1β, IL-6, Tumor necrosis factor-α (TNF-α) or Transforming growth factor-β (TGF-β) can augment APP expression\cite{4,5} and Aβ formation.\cite{6}

Inflammatory mediators present in AD lesions are thought to stimulate underlying key events of the pathological cascade that result in increased Aβ production with recruitment and activation of microglial cells. Microglias are an important source of PGs and they represent a suitable target for the activity of NSAIDs within the brain. Moreover, McGeer and Rogers proposed possible therapeutic effects of anti-inflammatory agents on the patients with AD.\cite{7}

John Robert Vane determined that administration of aspirin decreased prostaglandin synthesis, thus implicating prostaglandins as crucial in the inflammatory pathway. 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.\cite{8}

Severe degeneration of cholinergic neurons projecting from basal forebrain to cortical and hippocampus areas is one of the most basic and steady features of AD, among 90% loss of
basal forebrain cholinergic neurons has been found in AD patients. In contrast to the marked reduction of acetylcholine content in cholinergic target areas in AD brains, other transmitters such as serotonin, nor epinephrine and dopamine do not show a significant decrease.\cite{9}

The selective deficiency of acetylcholine (Ach) plays a major role in the genesis of the symptoms of AD. Therefore, a major approach to the treatment of AD involves attempts to augment the cholinergic function of the brain. This involves the use of inhibitors of acetyl cholinesterase such as tacrine, donepezil, rivastigmine and galantamine.

Hence this study was designed to evaluate the combination therapeutic influence of Aspirin along with Rivastigmine on scopolamine induced Alzheimer’s in rats to prevent the neuroinflammation progressed pathogenesis and improve the cholinergic transmission.

**MATERIALS AND METHODS**

**Experimental animals**

The healthy colony inbred mature Male Albino Wistar Rats, weighing 200-300gm were used in this study. The animals were kept under standard environmental conditions (temperature: 23 ± 2°C) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum. The animals were acclimatized under laboratory conditions three days prior to initiation of the experiment. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC) (Approval no: SVCP/IAEC/UG/2/02/2015 dated: 24.02.15).

**Experimental Design**

Four groups were employed in the present study, each comprising of six animals. The groups and treatment are designed as follows

Group 1: Vehicle control receives 0.5ml normal saline (NS), p.o.
Group 2: Alzheimer control receives scopolamine (1.0mg/kg/day i.p.).
Group 3: Rivastigmine (0.3mg/kg/day p.o.) + Scopolamine (1.0 mg/kg day i.p.)
Group 4: Aspirin (6.75 mg/kg/day p.o.) + Rivastigmine (0.3mg/kg/day p.o.)
+ Scopolamine (1.0 mg/kg/day i.p.).

**Induction of Alzheimer’s in rats**

Induction of AD in the rats was carried out by administration of Scopolamine intra peritonially at the dose of 1.0mg/kg body weight daily for 14 days. Completely trained
animals were chosen for this study.\textsuperscript{[10]} Body weight of each rats and daily feed consumption in all treatment groups were measured daily till continuation of the treatment.

**Cognitive and condition avoidance response**

All trained animals were tested on Pole climbing apparatus and Morris Water Maze for evaluating cognitive and condition avoidance response.

**Evaluation of Cognitive abilities**

Cognitive abilities were measured by using Morris Water Maze.\textsuperscript{[11]} The water maze consist of a circular tank with 100cm diameter and a wall 20cm above the water level. A circular platform (9cm diameter, covered with white line material for grip) is hidden 2cm below the water level. The water is made opaque using titanium dioxide suspension and is kept at about 23°C ± 2°C during experiment. Training places on the three consecutive days, with the rat receiving 4 consecutive trials per day with an inter-trial interval of 6-10min. Each trial is started from one of four assigned polar positions with a different sequence each day. The latency to find the platform is measured as the time of placement of the rat in the water to the time it finds the platform.

**Evaluation of Condition avoid response**

Condition avoidance response measured by the method of Fellow and Cook with some modifications using Cook’s pole climbing apparatus. Each trained rat was allowed to acclimatize for two minutes and was then exposed to a buzzer noise. After 5 seconds of putting on the buzzer (2.8 kHz), mild electric shocks (1.5 mA) 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 3 such trials were given to each rat at an interval of 1 min per day for 3 days. After about 3 days training schedule, most of the rats learned to climb the pole within average 26 seconds of starting the buzzer, thus avoiding the electric foot shocks. Rats avoiding the foot shocks in all 3 out of 3 trials were considered to have developed conditioned avoidance response for further experiments.\textsuperscript{[12]}

**Biochemical Analysis**

At end of the experimental period (14\textsuperscript{th} day) the animals were killed by decapitation the whole brain was rapidly removed weighed and homogenized in ice-cold phosphate buffer and prepared for biochemical estimation. Alzheimer’s rat brain biogenic amines Acetylcholine
(Ach)\textsuperscript{13} and Acetylcholinesterases (AchE)\textsuperscript{14} was estimated by Biochemical analysis method.

**Histopathological Examination**

All the animals were sacrificed at the end of the experiments. Hippocampus region of brain were removed of all the animals and post fixed in formal saline (24 hrs) washing was done in tap water then serial dilution (methyl, ethyl and absolute) were used to dehydration. Specimens were cleared in xylene and embedded in paraffin at 56\textdegree c in hot air oven for 24 hrs. Blocks were prepared by using paraffin wax at 4\textmu m thick in microtone. The obtained tissue section were deparaffinized and stained by hematoxylin and eosin (H&E) stain for histopathological examination through the light microscope.\textsuperscript{15}

**Statistical analysis**

The data represents as mean ± SEM of six replicated determinations. Results were analyzed statistically by one way ANOVA followed by post hoc Dunnett’s test by using SPSS V.17. The difference was considered significant when P<0.05.

**RESULTS**

**Changes in body weight**

The changes in body weight during the trial period was measured initial and final body weight changes in treatment group was compared with the control group (Group-I). On day 14\textsuperscript{th} final body weight of Group-II (Scopolamine 1mg/kg) only showed significant (P < 0.01) decrease in body weight when compared to the treatment group. Visually Group-III (Scopolamine 1mg/kg + Rivastigmine 0.3 mg/kg) (S+R) body weight was decreased when compared to the initial body weight of Group III as well as final weight of Group-I & IV (Table 1).

**Table 1: Changes in body weight**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Vehicle control)</td>
<td>266.67 ± 7.60</td>
<td>271.67 ± 6.01</td>
</tr>
<tr>
<td>Group – II (Scopolamine)</td>
<td>265.00 ± 8.47</td>
<td>250.00 ± 3.65\textsuperscript{**}</td>
</tr>
<tr>
<td>Group – III (S+R)</td>
<td>268.33 ± 7.92</td>
<td>258.33 ± 3.07</td>
</tr>
<tr>
<td>Group – IV (S+R+A)</td>
<td>266.67 ± 5.58</td>
<td>273.33 ± 4.94</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6
Symbols represent statistical significance: *** P<0.001, ** P <0.01, * P<0.05

Effect of Rivastigmine and Aspirin on feed intake in scopolamine induced Alzheimer’s rat

Table 2 illustrated that effect of Rivastigmine and Aspirin on feed intake in scopolamine induced Alzheimer’s rat, there is no significant different in feed intake between the treatment groups when compared to the vehicle control group.

Table 2: Effect of Rivastigmine and Aspirin on feed intake in scopolamine induced Alzheimer’s rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial feed intake (g)</th>
<th>Final feed intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Vehicle control)</td>
<td>65</td>
<td>90</td>
</tr>
<tr>
<td>Group – II (Scopolamine)</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>Group – III (S+R)</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>Group – IV (S+R+A)</td>
<td>65</td>
<td>85</td>
</tr>
</tbody>
</table>

Effect of Rivastigmine and Aspirin on Transfer latency in scopolamine induced Alzheimer’s rat by using Morris water maze test

On treatment day 1 there was no significant difference in transfer latency between the treatment groups when compare to the control groups. On day 7th scopolamine treated group shows significant (P < 0.01) increased latency when compared the vehicle control. In compare to the Group II (Scopolamine 1mg/kg) all the treatment showed significant decrease in transfer latency. Group I & II shows (P < 0.01) mild significant different and Group IV shows (P < 0.001) moderate significant different. On day 14th scopolamine treated group exhibited (P < 0.001) longer latency when compared the vehicle control. In compare to the Group II all the treatment showed significant (P < 0.001) decrease in transfer latency. These results suggest that rivastigmine with aspirin treatment effectively restored the cognitive deficits induced by Scopolamine (Table 3).
Table 3: Effect of Rivastigmine and Aspirin on Transfer latency in scopolamine induced Alzheimer’s rat using Morris water maze

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Vehicle control)</td>
<td>33.50 ± 1.98</td>
<td>28.33 ± 1.76&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>23.67 ± 1.52&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – II (Scopolamine)</td>
<td>38.83 ± 1.99</td>
<td>36.17 ± 2.36&lt;sup&gt;a**&lt;/sup&gt;</td>
<td>37.67 ± 1.84&lt;sup&gt;a***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – III (S+R)</td>
<td>33.33 ± 1.41</td>
<td>27.33 ± 1.23&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>24.00 ± 1.18&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – IV (S+R+A)</td>
<td>33.50 ± 2.45</td>
<td>25.50 ± 1.86&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>19.00 ± 1.46&lt;sup&gt;a***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6. Comparisons were made between:

a- Group I vs II, III and IV. b- Group II vs I, III and IV.

Symbols represent statistical significance: *** P<0.001, ** - P <0.01, *- P<0.05

Effect of Rivastigmine and Aspirin on Escape latency in scopolamine induced Alzheimer’s rat using Pole climbing Apparatus

Conditions avoid response on treatment day 1 there was no significant difference in escape latency between the treatment groups in compare to the vehicle control. On day 7 & 14 Group II showed significant (P < 0.001) increase in escape latency as compare to the Group I, in compare to the Group II; all the treatment showed significant (P < 0.001) decrease in escape latency (Table 4).

Table 4: Effect of Rivastigmine and Aspirin on Escape latency in scopolamine induced Alzheimer’s rat using Pole climbing Apparatus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Vehicle control)</td>
<td>25.00 ± 1.46</td>
<td>21.00 ± 1.06&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>16.50 ± 1.84&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – II (Scopolamine)</td>
<td>27.33 ± 1.92</td>
<td>30.33 ± 1.56&lt;sup&gt;a***&lt;/sup&gt;</td>
<td>26.33 ± 1.28&lt;sup&gt;a***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – III (S+R)</td>
<td>25.50 ± 1.20</td>
<td>20.17 ± 1.27&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>15.50 ± 2.66&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – IV (S+R+A)</td>
<td>25.50 ± 1.45</td>
<td>17.83 ± 1.26&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>12.83 ± 1.94&lt;sup&gt;a***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6. Comparisons were made between:

a- Group I vs II, III and IV. b- Group II vs I, III and IV.

Symbols represent statistical significance: *** P<0.001, ** - P <0.01, *- P<0.05
Protective effects of Rivastigmine and Aspirin on brain levels of acetylcholine (Ach) and acetylcholinesterase (AchE) in scopolamine induced Alzheimer’s rat

The results in table 5 showed the effect of treatment with rivastigmine & aspirin on cholinergic markers represent by brain Ach and AchE activity in scopolamine induced AD model. In comparison with vehicle control Group II (scopolamine 1mg/kg) produced significant (P < 0.001) reduction in brain Ach level, accompanied with significant increase in AchE level. The treatment groups showed significant (P < 0.001) increase in Ach and significant decrease in AchE activity.

Table 5: Protective effects of Rivastigmine and Aspirin on brain levels of acetylcholine and acetylcholinesterase in scopolamine induced Alzheimer’s rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Acetylcholine (Ach) (µmol/mg protein)</th>
<th>Acetylcholinesterase (AchE) (unit/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I (Vehicle control)</td>
<td>5.67 ± 0.27&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>0.42 ± 0.02&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – II (Scopolamine)</td>
<td>1.78 ± 0.18&lt;sup&gt;a***&lt;/sup&gt;</td>
<td>0.81 ± 0.03&lt;sup&gt;a***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – III (S+R)</td>
<td>3.40 ± 0.17&lt;sup&gt;a<em><strong>b</strong></em>&lt;/sup&gt;</td>
<td>0.58 ± 0.01&lt;sup&gt;a<em><strong>b</strong></em>&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group – IV (S+R+A)</td>
<td>4.43 ± 0.12&lt;sup&gt;a<em><strong>b</strong></em>&lt;/sup&gt;</td>
<td>0.47 ± 0.02&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6. Comparisons were made between:
- a- Group I vs II, III and IV.
- b- Group II vs I, III and IV.

Symbols represent statistical significance: *** P<0.001, ** - P <0.01, *- P<0.05

Histological investigation

Microscopic examination of brain sections of vehicle control group (Fig.1.a.) showed no histopathological alteration and normal histological structure of the hippocampus. Micrographs of brain section of AD-induced group showed severe neuronal degeneration with oedema (Fig.1.b.). The Rivastigmine alone treatment showed mild oedema (Fig.1.c.), Micrograph of brain section of AD-induced rats treated with rivastigmine along with low dose aspirin showing no histopathological alteration in the hippocampus (Fig.1.d).
DISCUSSION

In this study AD was induced by injecting scopolamine 1mg/kg for 14days. Scopolamine induced Aβ accumulation and oxidative stress. The neurotoxic effect of Aβ includes impairing synaptic plasticity, including apoptosis and promoting Tau phosphorylation and oxidative stress. In AD condition decrease in body weight due to the deficient absorption of nutrients through the intestine and weight loss may be associated with increased production of pro-inflammatory cytokines such as TNF-α and Interleukin-1. Treatment with Rivastigmine and Aspirin shows significant increase in body weight as compare to other treatment groups.
Morris water maze (MWM) was used to evaluate the effect of learning and memory improvement properties in rat. In MWM test observed that well trained animals in scopolamine injected group (Group II) showed significant transfer latency to find the platform which revealed scopolamine augment impairment of memory. Treatment groups (III&IV) showed significantly improves cognitive function as compare to scopolamine alone treated group. As compare between treatments rivastigmine and aspirin combination (Group-IV) shows more significant decrease in transfer latency.

In pole climbing test the impairment of learning and memory induced by scopolamine an anticholinergic agent, was reflected by increased time spent in shock zone. Active learning is a fundamental behaviour phenomenon. Treatment group showed that significant decrease in transfer latency which indicates that they are acting on Ach receptors because they had shown nootropic activity in presence of scopolamine which is a muscuranic receptor antagonist. In comparison between treatments rivastigmine and aspirin combination (Group-IV) shows more significant decrease in escape latency.

Scopolamine in adult male rats induced significant elevation of AchE and decrease in Ach, which reveals that decreased in cholinergic activity in scopolamine injected groups. Rivastigmine treatment produces significant increase in Ach level accompanied with significant decrease in AchE activity, also this result agrees with that of Liang and Tang. Aspirin along with rivastigmine also more significantly increase the Ach and decrease AchE activity.

Histopathological photograph of brain tissue section of AD rats showed neuronal degeneration, amyloid plaques and oedema. Rivastigmine alone treated group shows some oedema but rivastigmine along with aspirin combination shows no changes in histopathological photograph.

The results showed that aspirin and rivastigmine combination is effect in scopolamine induced Alzheimer’s. This combination more effectively preventing memory loss and improve the cognitive and condition avoid responses in AD induced rats. It also increases the cholinergic transmission, decreases AchE activity and decrease the Aβ plague formation as compare to the rivastigmine alone treated group.
CONCLUSION
It might be concluded that Rivastigmine combine with low dose aspirin have the profound beneficial effect on neuroinflammation progressed pathogenesis of Alzheimer’s and improve the cholinergic transmission in scopolamine induced alzheimer’s in rats. Further clinical data are required to explore this combination to improve the physical and psychological status of AD patients.

REFERENCE