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RESEARCH ARTICLE

Beneficial Effects of Statins on Experimental Amnesia

ATISH PRAKASH, NIRMAL SINGH and MANJEET SINGH

For author affiliations, see end of text.

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ABSTRACT

The present study was undertaken to investigate the beneficial effects of widely-prescribed lipid lowering drugs, pitavastatin, atorvastatin and simvastatin 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase inhibitors in cognitive dysfunctions of mice. Intra-cerebroventricular (ICV)-Streptozocin-(STZ)- and high-fat diet (HFD)-induced amnesia served as interoceptive memory models where as, Morris water-maze served as an exteroceptive model in the present study. A total of 13 groups, comprising seven mice in each group were used in this investigation. Day 4 Escape latency time (ELT) recorded during acquisition trials conducted from day 1 to day 4, in water-maze was taken as an index of acquisition, where as mean time spent in target quadrant during retrieval trial on day 5, was taken as an index of retrieval (memory). ICV-STZ-(3 mg kg $^{-1}$ i.p.), and HFD-treated (for 90 days) mice showed an impairment of acquisition as well as retention on water maze task as reflected by significant increase in ELT on day 4 and decrease in time spent in target quadrant on day 5. Pitavastatin (5 mg kg $^{-1}$), atorvastatin (5 mg kg $^{-1}$) and simvastatin (5 mg kg $^{-1}$) significantly attenuated ICV-STZ- and HFD-induced amnesia. These results highlight the ameliorative role of statins in experimental amnesia with possible involvement of their cholesterol-dependent as well as cholesterol independent actions.

Keywords: *Statins, Cholesterol, Memory, Water maze, Amnesia, Beta-amyloid*

There has been a rise in the number of patients suffering from Alzheimer's disease (AD) all over the world. Dementia is a common feature of AD, cerebrovascular diseases (multi infarct dementia) and other conditions primarily or secondarily affecting the brain. It has been observed that, hypertension, history of stroke, diabetes mellitus and hypercholesterolaemia are all associated with high risk of AD, providing evidence of great overlap between AD and vascular dementia [1]. The main histological features of AD include extracellular deposition of β-amyloid (Aβ) plaques and intraneuronal neurofibrillary tangles. Many clinical studies have suggested that the net brain cholesterol concentration is regulated by serum cholesterol level and there is a cross talk between the CNS and peripheral cholesterol pools [2, 3]. Cholesterol turnover appears to play a crucial role in the deposition and clearance of amyloid peptide in brain. Furthermore, serum cholesterol, atherosclerosis, apolipoprotein-E and AD all appear to be interconnected [4, 5, 6]. ApoE is a cholesterol transporting protein that is associated with amyloid deposits [7]. Elevated serum cholesterol levels not only lead to atherosclerosis but also carry a high risk of developing AD [8]. High cholesterol levels appear to be intimately associated with development of amyloid plaques in humans [9, 10]. Epidemiological studies revealed that individuals with high peripheral cholesterol levels show more susceptibility to Alzheimer's disease [4, 5, 6, 11], and the incidence of AD is higher in countries with high-fat and high-calorie diets [12]. Moreover experimental studies have shown that cholesterol fed wild type rabbits develop brain pathology similar to that of AD [4]. Transgenic mouse model of AD exhibited increased Aβ plaques when mice were fed with cholesterol rich diet [14]. Cell culture and in vivo animal studies have shown that reducing cholesterol can inhibit Aβ synthesis [13, 14]. Degeneration that occurs in AD is mediated through modulation of cholesterol signaling in the brain [15]. Therefore, a new approach aimed at controlling blood cholesterol level is gathering momentum for the management of AD. Statins, the 3-hydroxy-3-methylglutaryl-coenzyme-A reductase inhibitors (HMG CoARI) are widely prescribed drugs in the world for their cholesterol lowering action [16]. They are com-

Table 1. Effect of High fat diet on body weight of Normal diet control

Group / Treatment	Day 1, mean body weight (g)	Day 30, mean body weight (g)	Day 60, mean body weight (g)	Day 90, mean body weight (g)
control (normal diet)	20.4 ± .7	22.6 ± 1.3	23.6 ± 1.0	24.4 ± 1.2 [a]
High fat diet,	20.6 ± 1.1	25.1 ± 1.1	27.4 ± 1.2	29.9 ± 1.3 [a b]

Each group (n=7) represents mean ± S.E.M
[a] denotes $p<0.05$ compared to d 1 body weight
[b] denotes $p<0.05$ compared to d 90 body weight in control(normal diet).
t-test

monly used for the treatment of dyslipidemias. Recent reports indicate that statins produce many effects which are independent of their cholesterol lowering property.

These pleotropic effects of Statins include anti-thrombotic, anti-inflammatory, anti-oxidative actions, improvement of endothelial dysfunction, vasodilatation, atherosclerotic plaque stabilization, anti proliferative & immunosuppressive effects [17, 18]. Studies carried out with cultured rat cortical neurons have reported neuro-protective actions of Statins against glutamate induced excitotoxicity [19]. There are controversial reports regarding the effect of statins on cognitive function. Although, there are a few studies showing cognitive decline [20, 21, 22], some studies showing no effect on memory [23, 24] but several studies suggest improvement of cognitive functions with statin therapy [25, 26]. Hence cognition modulatory role of statins deserves further investigation therefore, the present study was designed to investigate the role of statins (Pitavastatin, Simvastatin & Atorvastatin) in high fat diet (HFD) and intracerebroventricular streptozotocin (ICV STZ) induced amnesia in mice employing Morris water-maze test.

MATERIAL AND METHODS

Swiss albino mice (20-30 gm) of either sex (procured from IVR, Izatnagar, India) were employed in the present study and were housed in animal house with free access to water and standard laboratory pellet chow diet (Kisan Feeds Ltd, Mumbai, India). The high fat diet (HFD) groups of animals were subjected to standard diet enriched with fat *ad libitum* for 90 days. The mice were exposed to12 h light and 12 h dark cycle. The experiments were conducted between 10.00 to 17.30 h in a semi sound-proof laborotary. The animals were acclimatized to the laboratory condition five days prior to behavioural study. Experimental, protocol was approved by the Institutional Animal Ethics Committee (IAEC). Care of the animals was taken as per guidelines of CPCSEA, ministry of Forests and environment, Government of India (Reg. No107).

Drugs

All the drug solutions and suspensions were freshly prepared before use. Pitavastatin (Zydus Research Center, Ahmedabad), Atorvastatin (Zydus Research Center, Ahmedabad) and Simvastatin (Morpen, Baddi, HP) were suspended in 1% w/v of sodium carboxy methyl cellulose (CMC). Streptozotocin (Sigma Chemicals) was dissolved in artificial cerebrospinal fluid (CSF) (147mM Nacl; 2.9mM KCl; 1.6mM $MgCl_2$; 1.7 mM $CaCl_2$; and 2.2 mM dextrose). Standard Cholesterol estimation kit (Monozyme India Limited, Secunderabad) was used to estimate total serum cholesterol level.

Laboratory models

Exteroceptive model; Morris water-maze apparatus (MWM)

Morris Water Maze [27] is the most commonly used model to test memory. The MWM procedure was based on a principle where the animal was placed in a large pool of water, as animal dislike swimming, their tendency was to escape from the water being accomplished by finding an escape platform. MWM consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1° C). The water was made opaque with white colored dye. The tank was hypothetically divided into four equal quadrants with help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform ($10cm^2$), painted white was placed inside the target quadrants of this pool, 1cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trials on each day with gap of 5 min. The mouse was gently placed in the water of the pool between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 s to locate

Table 2. Effect of Pitavastatin / Simvastatin / Atorvastatin on escape latency time (ELT) of control and high fat diet (HFD) Mice

Group	Treatment	Dose /Kg^{-1}	Day 1 ELT in sec	Day 4 ELT in sec
I	Control (1% w/v CMC)	10 ml p.o.	91 ± 1.7	43.72 ± 1.7 [a]
II	Pitavastatin	5 mg p.o.	88.58 ± 1.7	38 ± 1.7
III	Simvastatin	5 mg p.o.	87.55 ± 1.6	40 ± 1.6
IV	Atorvastatin	5 mg p.o.	91.25 ± 1.8	38.93 ± 1.8
V	HFD	90 days	91.12 ± 3.3	62.56 ± 3.3 [b]
VI	HFD + Pitavastatin	5 mg p.o.	88.58 ± 2.9	38 ± 2.9 [c]
VII	HFD + Simvastain	5 mg p.o.	88.5 ± 3	40.75 ± 3 [c]
VIII	HFD + Atorvastain	5 mg p.o.	87.2 ± 2.7	39.41 ± 2.7 [c]

Each group (n=7) represents mean ± S.E.M
[a] denotes $p < 0.05$ as compared to day 1 ELT in control.
[b] denotes $p < 0.05$ as compared to day 4, ELT in control.
[c] denotes $p < 0.05$ as compared to day 4, ELT in HFD treatment group.
ANOVA followed by Tukey's multiple range test.

Table 3. Effect of Pitavastatin / Simvastatin / Atorvastatin on escape latency time (ELT) of control (intracerebroventricular cerebrospinal fluid) and intracerebroventricular (ICV) Streptozotocin (STZ) treated Mice

Group	Treatment	Dose /Kg^{-1}	Day 1 ELT in sec	Day 4 ELT in sec
I	Control (1% w/v CMC)	10 ml p.o.	91 ± 1.7	43.72 ± 1.7 [a]
II	Pitavastatin	5 mg p.o.	88.58 ± 1.7	38 ± 1.7
III	Simvastatin	5 mg p.o.	87.55 ± 1.6	40 ± 1.6
IV	Atorvastatin	5 mg p.o.	91.25 ± 1.8	38.93 ± 1.8
X	STZ	3 mg, 10 µl i.c.v	106.25 ± 3.3	76.28 ± 3.3 [b]
XI	STZ + Pitavastatin	5 mg p.o.	93.55 ± 2	40.06 ± 2 [c]
XII	STZ + Simvastain	5 mg p.o.	91.45 ± 2.3	39.5 ± 2.3 [c]
XIII	STZ + Atorvastain	5 mg p.o.	91.75 ± 2.5	40.5 ± 2.5 [c]

Each group (n=7) represents mean ± S.E.M
[a] denotes $p < 0.05$ as compared to day 1 ELT in control.
[b] denotes $p < 0.05$ as compared to day 4, ELT in control.
[c] denotes $p < 0.05$ as compared to day 4, ELT in STZ treatment group.
ANOVA followed by Tukey's multiple range test.

submerged platform. Then, it was allowed to stay on the platform for 20 s. If it failed to find the platform within 120 s, it was guided gently onto platform and allowed to remain there for 20 s. Escape latency time (ELT) to locate the hidden platform in water maze was noted as index of acquisition or learning. Animal was subjected to acquisition trials for four consecutive days. On fifth day, platform was removed and each mouse was allowed to explore in the pool for 120 s. Mean time spent in all four quadrants was noted. The time spent by the animal in target quadrant searching for the hidden platform is noted as index of retrieval. ELT and time spent by the animals in quadrants were recorded manually with the help of a digital recording device having four separate stop watches.

Acquisition Trial

Each mouse was subjected to four trials on each day. A rest period of 5 min was allowed in between each trial. Four trials per day were repeated for four consecutive days. Starting position on each day to conduct four acquisition trials was changed as described below and Q4 was maintained as target quadrant in all acquisition trials.

Day1	Q1	Q2	Q3	Q4
Day2	Q2	Q3	Q4	Q1
Day3	Q3	Q4	Q1	Q2
Day4	Q4	Q1	Q2	Q3

Mean escape latency time (ELT) calculated for each day during acquisition trials and day 4 ELT was used as an index of acquisition.

Retrieval Trial

On fifth day the platform was removed. Mouse was placed in water maze and allowed to explore the maze for 120 s. Each mouse was subjected to four such trials and each trial was started from different quadrant. Mean time spent in all three quadrants i.e. Q1, Q2 and Q3 were recorded and the time spent in the target quadrant i.e. Q4 in search of missing platform provided an index of retrieval. Care was taken to ensure that the relative location of water maze with respect to other objects in the laboratory serving as prominent visual clues was not disturbed during the total duration of study.

Interoceptive models; (a) Streptozotocin (STZ) induced amnesia **(b)** High fat diet (HFD) induced amnesia

Streptozotocin (STZ), intra cerebroventricular (ICV) induced amnesia

Mice were anesthetised with anesthetic ether [28] . A polypropylene tube was placed round a hypodermic needle of 0.4 mm external diameter exposing about 3mm at the tip, and was attached to a 10µl Hamilton microlitre syringe (Top Syringe, Mumbai, India), which was inserted perpendicularly through the skull (not more than 3mm) into the brain of mouse.The injection site was 1mm to right or left midpoint on the line drawn through to the anterior base of the ears. Injections were performed into right or left ventricle randomly. To ascertain that the drug was administered exactly into the cerebral ventricles, some mice (20%) were injected with 5µl of diluted potent blue dye and their brains were examined macroscopically after sectioning. STZ was dissolved in artifical CSF (25 mg ml $^{-1}$) solution which was made freshly just before the injection. The STZ (3 mg kg $^{-1}$) was given ICV injection bilaterally in two divided doses, on the first and the third day. The concentration was adjusted so as to deliver 10 µl at a site. Control group mice were given ICV injection of artifical CSF (147 mM NaCl; 2.9 mM KCl; 1.6 mM MgCl2; 1.7 mM CaCl2; and 2.2mM dextrose) on first and third day.

High fat diet (HFD) induced amnesia

Animals were subjected to cholesterol rich diet (HFD) for 90 days to induce amnesia.

Estimation of serum total cholesterol

Blood sample was withdrawn from retro orbit sinus. The sample was allowed to clot in Eppendorf for half an hour. Then centrifuged for 15 minutes at 4000 rpm to separate the serum form clot debris. The total serum cholesterol levels were estimated by Allain method with slight modifications by employing commercially available standard cholesterol estimation kit [29]. The absorbance was measured against blank at 540nm spectrophotometrically.

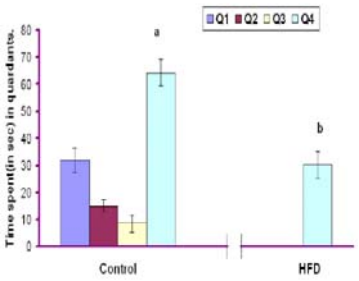

Fig 1: Effect of High Fat Diet (HFD) on mean time spent in target quadrant during day 5 retrieval trial on water-maze. Values are expressed as mean ± S. E. M. (n=7). a = $p < 0.05$ Vs time spent in other quadrants in control group. b = $p < 0.05$ Vs time spent in target quadrant i.e. Q-4 in control group.

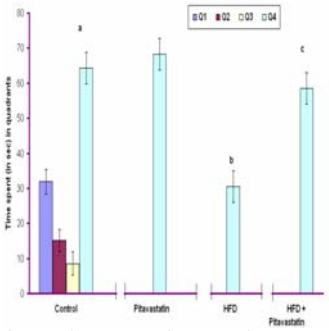

Fig: Effect of Pitavastatin on mean time spent in target quadrant of Normal and High Fat Diet (HFD) Mice, during day 5 retrieval trial on water-maze. Values are expressed as mean ± S.E.M. (n=7). a = $p < 0.05$ Vs time spent in other quadrants in control group. b = $p < 0.05$ Vs time spent in target quadrant i. e., Q-4 in control group. c = $p < 0.05$ Vs time spent in target quadrant i.e., Q-4 in HFD treated group.

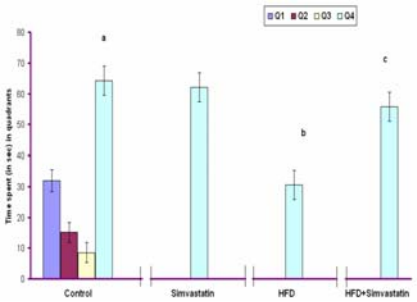

Fig 3: Effect of Simvastatin on mean time spent in target quadrant of Normal and High Fat Diet (HFD) Mice, during day 5 retrieval trial on water-maze. Values are expressed as mean ± S.E.M. (n=7). a = $p < 0.05$ Vs time spent in other quadrants in control group. b = $p < 0.05$ Vs time spent in target quadrant i.e., Q-4 in control group. c = $p < 0.05$ Vs time spent in target quadrant i.e., Q-4 in HFD treated group.

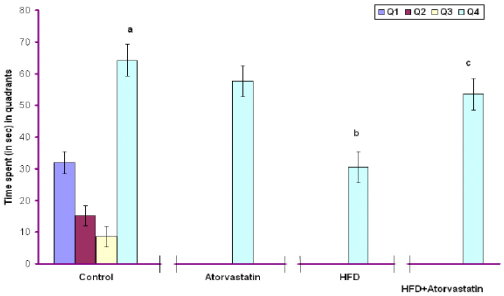

Fig 4: Effect Atorvastatin on mean time spent in target quadrant of Normal and High Fat Diet (HFD) Mice, during day 5 retrieval trial on water-maze. Values are expressed as mean ± S.E.M. (n=7). a = $p < 0.05$ Vs time spent in other quadrants in control group. b = $p < 0.05$ Vs time spent in target quadrant i. e. Q-4 in control group. c = $p < 0.05$ Vs time spent in target quadrant i.e. Q-4 in HFD treated group.

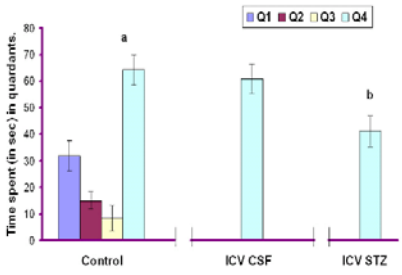

Fig 5: Effect of Vehicle [intracerebroventricular (ICV) cerebrospinal fluid (CSF)] and ICV Streptozotocin (STZ) on mean time spent in target quadrant during day 5 retrieval trial on water-maze. Values are expressed as mean ± S.E.M. (n=7). a = $p < 0.05$ Vs time spent in other quadrants in control group. b = $p < 0.05$ Vs time spent in target quadrant i.e. Q- 4 in control group.

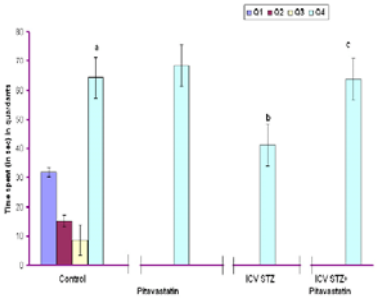

Fig 6: Effect of Pitavastatin on mean time spent in target quadrant of control (ICV CSF) and ICV Streptozotocin (STZ) treated Mice, during day 5 retrieval trials on water-maze. Values are expressed as mean ± S.E.M. (n=7). a = $p < 0.05$ Vs time spent in other quadrants in control group. b = $p < 0.05$ Vs time spent in target quadrant i.e. Q-4 in control group. c = $p < 0.05$ Vs time spent in target quadrant i.e. Q-4 in STZ treated group.

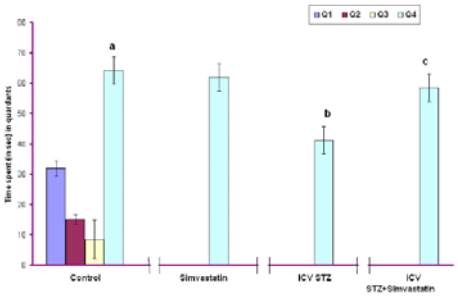

Fig 7: Effect of Simvastatin on mean time spent in target quadrant of control (ICV CSF) and ICV Streptozotocin (STZ) treated Mice, during day 5 retrieval trials on water-maze. Values are expressed as mean ± S.E.M. (n=7). a = $p < 0.05$ Vs time spent in other quadrants in control group. b = $p < 0.05$ Vs time spent in target quadrant i.e. Q-4 in control group. c = $p < 0.05$ Vs time spent in target quadrant i.e. Q-4 in STZ treated group.

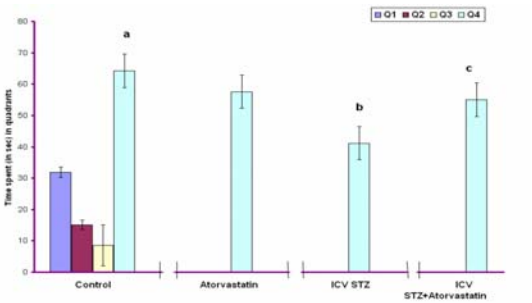

Fig 8: Effect of Atorvastatin on mean time spent in target quadrant of control (ICV CSF) and ICV Streptozotocin (STZ) treated Mice, during day 5 retrieval trial on water-maze. Values are expressed as mean ± S.E.M. (n=7). a = $p < 0.05$ Vs time spent in other quadrants in control group. b = $p < 0.05$ Vs time spent in target quadrant i.e. Q-4 in control group. c = $p < 0.05$ Vs time spent in target quadrant i.e. Q-4 in STZ treated group.

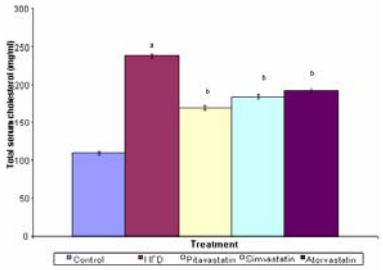

Fig 9: Effects of Pitavastatin, Simvastatin and Atorvastatin on total serum Cholesterol. Values are expressed as mean ± S. E. M. a = $p < 0.05$ Vs total serum cholesterol level of control group. b = $p < 0.05$ Vs total serum cholesterol level in HFD mice.

Experimental Protocol

Thirteen groups of mice were employed in the present study and each group comprised of seven mice.

Group I (normal diet fed vehicle treated group), n = 7

The mice were subjected to normal diet for 90 days before acquisition (91st day to 94th day) and retrieval test (95th day) on water maze. These mice were administered vehicle (1% w/v CMC, 10 ml kg $^{-1}$ p.o.) 30 min prior to the acquisition trials and before retrieval test.

Groups II, III & IV (Pitavastatin / Simvastatin / Atorvastatin per se group), n=7

Mice were administered Pitavastatin (5mg kg^{-1} p.o.) / Simvastatin / Atorvastatin daily for 15 days and again for four consecutive days (day1 to day 4, 30 min before) during acquisition trials. On day 5 the animals were administered vehicle (1% w/v CMC, 10ml kg^{-1} p.o.) 30 min before retrieval trial.

Group V (High Fat Diet Treated group), n=7

Mice were administered high fat diet for 3 months (90 days). HFD mice were exposed to the water maze for four consecutive days during acquisition trials (Day 1 to Day 4) and retrieval trial was carried out on day 5.

Group VI (HFD + Pitavastatin Treated Group), n=7

HFD mice, were administered pitavastatin (5mg kg^{-1} p.o.) suspended in 1% w/v CMC, daily for 15 days and again for four consecutive days (day1 to day4, 30 min before) during acquisition trials. On day 5 these HFD mice were administered vehicle (1% w/v CMC, 10ml kg^{-1} p.o.) 30 min before retrieval trial.

Group VII (HFD + Simvastatin Treated Group), n=7

HFD mice were administered simvastatin (5mg kg^{-1} p.o.) suspended in 1% w/v CMC, daily for 15 days and again for four consecutive days (day1 to day4, 30 min before) during acquisition trials. On day 5 these HFD animals were administered vehicle (1% w/v CMC, 10ml kg^{-1} p.o.) 30 min before retrieval trial.

Group VIII (HFD + Atorvastatin Treated Group), n=7

HFD mice were administered atorvastatin (5mg kg^{-1} p.o.) suspended in 1% w/v CMC, daily for 15 days and again for four consecutive days (day1 to day4, 30 min before) during acquisition trials. On day 5 these HFD mice were administered vehicle (1% w/v CMC, 10ml kg^{-1} p.o.) 30 min before retrieval trial.

Group IX (ICV CSF Treated Control Group), n=7

Mice were injected artificial cerebro-spinal fluid, (25 mg ml^{-1}, 10µl, i.c.v.) in two doses schedules i.e. on 1st and on 3rd day.

Group X (ICV STZ Treated Group), n=7

Mice were injected streptozotocin (3mg kg^{-1}, 10µl, i.c.v.) in two doses schedules i.e. on1st and on 3rd day.

Group XI (ICV STZ + Pitavastatin Treated Group), n=7

ICV STZ mice were administered pitavastatin (5mg kg^{-1} p.o.) suspended in 1% w/v CMC, daily for 15 days and again for four consecutive days (day1 to day4, 30 min before) during acquisition trials. On day 5 the mice were administered vehicle (1% w/v CMC, 10ml kg^{-1} p.o.) 30 min before retrieval trial.

Group XII (ICV STZ + Simvastatin Treated Group), n=7

ICV STZ mice were administered Simvastatin (5mg kg^{-1} p.o.) suspended in 1% w/v CMC, daily for 15 days and again for four consecutive days (day1 to day4, 30 min before) during acquisition trials. On day 5 the animals were administered vehicle (1% w/v CMC, 10ml kg^{-1} p.o.) 30 min before retrieval trial.

Group XIII (ICV STZ + Atorvastatin Treated Group), n=7

ICV STZ mice were administered Atorvastatin (5mg kg^{-1} p.o.) suspended in 1% w/v CMC, daily for 15 days and again for four consecutive days (day1 to day4, 30 min before) during acquisition trials. On day 5 the mice were administered vehicle (1% w/v CMC, 10ml kg^{-1} p.o.) 30 min before retrieval trial.

Statistical Analysis

All results were expressed as mean ± S.E.M (standard error of mean). Data was analyzed using one way ANOVA followed by *post hoc* Tukey's test and Bonferroni test using SigmaStat Statistical Software, version 2.0. $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of high fat diet on body weight of mice

There was a significant increase ($p < 0.05$) in the body weight of animals over the period of 90 days in mice receiving normal diet or high fat diet (HFD), when compared to the body weights of mice on day 1. Furthermore, HFD treatment for 90 days produced a sig-

nificant increase ($p < 0.05$) in body weight of mice as compared to those receiving normal diet for 90 days (Table1).

Effect of Acquisition and Retrieval Trials on Escape Latency Time (ELT) and Time Spent in Target Quadrant (TSTQ)

Administration of vehicle (1% w/v CMC, 10 ml kg^{-1} p.o.) 30 min before acquisition trials conducted on day 1 to day 4, significantly decreased day 4 escape latency time (ELT) as compared to its value noted on day 1 (Table-2). Further, these mice during retrieval trial conducted on day 5 spent, significantly more time in the target quadrant (Q4) in search of missing platform as compared to the time spent in other quadrants (Q1, Q2, Q3) on water-maze (Fig 1).

Effect of High Fat Diet (HFD) on Acquisition and Retrieval of Memory

Mice that were subjected to high fat diet for 3 months exhibited a significant increase in day 4ELT as compared to day 4 ELT of control group (Table-2). HFD treatment also significantly, decreased time spent in target quadrant (TSTQ) in search of missing platform during retrieval trial conducted on day 5. Impairment of acquisition due to HFD may have led to failure of retrieval (Fig 1).

Effect of Statins on Acquisition and Retrieval of Memory in HFD Mice

Administration of Pitavastatin (5mg kg^{-1} p.o.) / Simvastatin (5mg kg^{-1} p.o.) / Atorvastatin (5mg kg^{-1} p.o.) daily for 15 days and 60 min before acquisition trials conducted on day 1 to day 4 significantly prevented HFD induced increase in day 4 ELT (Table-2). They also attenuated decrease in time spent in target quadrant (Q4) in HFD animals, in search of missing platform during retrieval trial conducted on day 5. These observations indicated reversal of HFD induced amnesia by statins (Fig 2; Fig 3; Fig 4).

Effect of Streptozotocin (STZ) on Acquisition and Retrieval of Memory

Administration of artificial CSF (25 mg ml-1, 10µl, i.c.v.) did not produce any significant effect on decrease in day 4 ELT of control group (Table-3) and increase in time spent in the target quadrant (Q4) in search of missing platform during retrieval trial conducted on day 5 (Fig 5). Streptozotocin (3 mg kg-1, 10µl, i.c.v.) dissolved in artificial CSF solution significantly attenuated the decease in day 4 ELT during acquisition trials as compared to control group (Table-3). Further, it markedly reduced the time spent in target quadrant (Q4) in search of missing platform during retrieval trial conducted on day 5 (Fig 5). These findings suggested that streptozotocin had produced impairment of acquisition as well as retrieval of memory.

Effect of Statins on Streptozotocin (STZ) induced Amnesia

Administration of pitavastatin (5mg kg^{-1} p.o.) / simvastatin (5mg kg^{-1} p.o.) / atorvastatin (5mg kg-1 p.o.) to STZ treated mice, significantly prevented increase in STZ induced day 4 ELT during acquisition trials (Table-3). Treatment with the statins also prevented STZ induced decrease in time spent in target quadrant (Q4) in search of missing platform during retrieval trial suggesting that statins had reversed STZ induced amnesia in mice (Fig 6; Fig 7; Fig 8).

Effect of Statins on Total Serum Cholesterol

Mice subjected to high fat diet for 3 months showed a significant increase in their total serum cholesterol levels, when compared to control group. Pitavastatin, simvastatin and atorvastatin treatment for 15 days produced a significant fall in total serum cholesterol level in HFD mice (Fig 9).

DISCUSSION

Morris Water Maze, [27, 30] is employed in the present study as an exteroceptive model to evaluate spatial learning and memory. Extensive pre training is not required in this model because animals learn rapidly to locate the hidden platform. Moreover escape from water itself acts as motivation and eliminates the use of other motivational stimuli such as food and water deprivation. Water provides a uniform environment and eliminate the interference due to olfactory clues [27, 30]. Motor in-coordination alters swimming ability of animal and any effect due to impaired motor coordination may be easily detected in this experimental model.

A marked decrease in escape latency time (ELT) during ongoing acquisition trials denote normal acquisition of memory and an increase in time spent in target quadrant in search of missing platform during retrieval trial indicates retrieval of memory. These observations are in agreement with the results of our earlier studies and reports from other laboratory [31]. The vehicles used to prepare the various solutions of drugs produced no significant effect on memory acquisition and retrieval, showing that the drugs were responsible for the observed changes in memory.

A significant rise in body weight of mice was observed after 90 days of normal diet / HFD administration. Furthermore, mice subjected to HFD for 90 days produced a significant rise in body weight, when compared to those fed for 90 days with normal diet. The swimming ability or driving motivation to the platform was not altered despite increased body weight of rats on 90th day, since there was no significant variation in the ELT of mice recorded on 91st day (whether receiving normal diet or HFD). In other words, changes in body weight did not interfere with the swimming ability of mice in any way.

In the present study chronic administration (3 months) of high fat diet (HFD) has produced significant increase in total cholesterol and impairment of learning and memory. In animal studies, dietary cholesterol ac-

celerates deposition of amyloid β peptide in brain. Increase in brain cholesterol is documented to enhance deposition of amyloid β peptide [10]. β-amyloid deposits in brain exert neurotoxic action and are instrumental in the formation of senile plaque in AD. Senile plaque is the focus of a complex cellular reaction involving the activation of both microgalia and astrocytes adjacent to amyloid plaque. β-amyloid induced activation of microgalia results in synthesis and secretion of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α [32]. In addition β-amyloid has been shown to affect a variety of neuronal functions including calcium signaling and impairment of mitrochondrial redox activity [33, 34]. All these effects of β-amyloid may eventually lead to neuronal damage and dementia. Moreover mature rats fed with adequate diet, having higher amounts of fat show cognitive deficits [35, 36, 37]. HFD induced cognitive deficit can be overcome with cholesterol lowering agents [36]. This view is further supported by our observation, that statins reversed HFD induced amnesia probably by producing a significant fall in total serum cholesterol level or through their anti-inflammatory and anti-oxidant actions.

In our study, streptozotocin (ICV STZ) at subdiabetogenic doses significantly impaired learning and memory in mice. The ICV STZ model has been described as an appropriate animal model for dementia of AD, typical feature of which is progressive impairment of memory [38, 39]. After ICV administration, the highest concentration of STZ (3mg kg^{-1}) reached the fornix and periventricular white matter at the level of 3rd ventricle, which showed the greatest damage [40]. ICV STZ induced amnesia was independent of its hyperglycemic effect [41]. Although the mechanism of action of STZ on memory impairment is not yet known, it probably involves the induction of oxidative stress [42, 43], to which myelin is particularly vulnerable [44]. Damage to myelin by oxidative stress is seen in disorders such as AD with cognitive impairment [45]. ICV STZ in rats causes desensitization of insulin receptors and biochemical changes similar to that of AD or ageing brain [46, 47, 48]. In addition, reduced energy metabolism and synthesis of acetyl CoA ultimately results in cholinergic deficiency and thereby memory deficit in ICV STZ treated rats. In our recent investigation ICV STZ significantly enhanced brain acetyl cholinesterase (AChE) activity, brain thiobarbituric acid reactive species (TBARS) levels and reduced brain glutathione (GSH) levels of rats along with cognitive decline when tested on Morris water-maze [49]. Statins are known to exert potential neuroprotective action by virtue of their anti-inflammatory and anti-oxidative properties [50, 51, 52]. Pitavastatin in our recent study significantly decreased brain AChE activity, TBARS levels and prevented decrease in brain GSH levels [49]. Therefore, reversal of ICV STZ induced amnesia by statins may involve this potential anti-oxidative, anti-inflammatory, anti-AChE activity and neuroprotective action. We have shown that Pitavastatin, Simvastatin and Atorvastatin are able to reverse HFD and STZ induced amnesia in mice probably by virtue of their cholesterol dependent as well as cholesterol independent actions. Nevertheless further studies are needed to unearth the exact mechanism of memory improving effect of statins.

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REFERENCES

1. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993; 261: 921-23.
2. Bjorkhem I, Lutjohann D, Diczfalusy U, Stahle L, Ahloborg G, Wahren D. Cholesterol homeostasis in the human brain: Turnover of 24S-hydoxycholesterol and evidence for a cerebral origin of most oxysterol in the circulation. *J Lipid Res*. 1998; 39:1594-00.
3. Haley RW, Dietschy JM. Is there a connection between the concentration of cholesterol circulating in the plasma and the rate of neuritic plaque formation in Alzheimer's disease? *Arch Neurol*. 2000; 57: 1410-12.
4. Jarvik GP, Wijsman EM, Kukull WA, Schellenberg GD, Yu C, Larson EB1. Interaction of apolipoprotein E genotype, total cholesterol level and sex in prediction of Alzheimer disease in a case-control study. *Neurology*. 1995; 45:1092-96.
5. Notkola IL, Sulkava R, Pekkanen J, Erkinjuntti T, Ehnholm C, Kivinen P, Tuomilehto J, Nissinen A. Serum cholesterol, apolipoprotein E epsilon 4 allele and Alzheimer's disease. *Neuroepidemiology*. 1998; 17: 14-20.
6. Roher AE, Kuo YM, Kokjohn KM, Emmerling MR, Gracon S. Amyloid and lipids in the pathology of Alzheimer disease. *Amyloid International J Exp Clin Investigation*. 1999; 6: 136-45.
7. Poirier J. Apolipoprotein-E, Cholesterol transport and synthesis in sporadic Alzheimer's disease. *Neurobiol Aging*. 2005; 26: 355–61.
8. Spark DL. Coronary artery disease, hypertension, ApoE, and cholesterol: a link to Alzheimer's disease? *Cerebrovascular pathol Alzheimer's dis*.1997; 826: 128-46.
9. Bales KR, Verina T, Dodel RC, Du Y, Altstiel L, Bender M, Hyslop P, Johnstone EM, Little SP, Cummins DJ, Piccardo P, Ghetti B, Paul SM. Lack of apolipoprotein E dramatically reduces amyloid β-peptide deposition. *Nat Genet*.1997; 17: 263-64.
10. Emma RF, David JS, Claire EW, Huw D, Brian MA. The role of cholesterol in biosynthesis of β-amyloid. *Neuro Report*. 1999; 10: 1699-05.
11. Spark DL, Scheff SW, Hunsaker JC, Liu H, Landers T, Gross DR. Induction of Alzheimer's like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol*. 1994; 126: 88-94.
12. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MMB. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Ann Neurol*. 1997; 42: 776-82.
13. Sparks DL, Connor DJ, Browne PJ, Lopez JE, Sabbagh MN. HMG-CoA reductase inhibitors(statins) in the treatment of Alzheimer's disease and why it would be ill-advised to use one that crosses the blood-brain barrier. *J Nutr Health Aging*. 2002; 6: 324-31.
14. Golde TE, Eckman CB. Cholesterol modulation as an emerging strategy for the treatment of Alzheimer's disease. *DDT*. 2001; 6: 1049-55.

15. Shobab LA, Hsiung GR, Feldman HH. Cholesterol in Alzheimer's disease. *Lancet Neurol*. 2005; 4: 841–52.
16. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature*. 1990; 343: 425–30.
17. Liao James K, Laufs U. Pleiotropic effects of statins. *Annu Rev Pharmacol Toxicol*. 2005; 45: 89-18.
18. Vaughan CJ. Prevention of Stroke and Dementia with Statins: Effects beyond Lipid Lowering. *Am J Cardiol*. 2003; 91: 23B–9, suppl
19. Bosel J, Gandor F, Harms C, Synowitz M, Harms U, Djoufack PC, Megow D, Dirnagl U, Hortnagl H, Fink KB, Endres M. Neuroprotective effects of atorvastatin against glutamate induced excitotoxicity in primary cortical neurons. *J Neurochem*. 2005; 92 (6): 1386-98.
20. Wagstaff LR, Mitton MW, Arvik BM, Doraiswamy PM. Statin-associated memory loss: analysis of 60 case reports and review of literature. *Pharmacotherapy*. 2003; 23(7): 871- 80.
21. Galatti L, Polimeni G, Salvo F, Romani M, Sessa A, Spina E. Short-term memory loss associated with rosuvastatin. *Pharmacotherapy*. 2006; 26(8):1190-02.
22. Padala KP, Padala PR, Potter JF. Simvastatin-induced decline in cognition. *Ann Pharmacother*. 2006; 40(10):1880-3.
23. Baytan SH, Alkanat M, Ozeren M, Ekinci M, Akgun A Fluvastatin alters psychomotor performance and daily activity but not the spatial memory in rats. *Tohoku J Exp Med*. 2006; 209(4):311-20.
24. Muldoon MF, Barger SD, Ryan CM, et al. Effects of lovastatin on cognitive function and psychological well-being. *Am J Med*. 2000; 108, 538-46.
25. Li L, Cao D, Kim H, Lester R, Fukuchi K. Simvastatin enhances learning and memory independent of amyloid load in mice. *Ann Neurol*. 2006; 60(6):729-39.
26. Parale GP, Baheti NN, Kulkarni PM, Panchal NV. Effects of atorvastatin on higher functions. *Eur J Clin Pharmacol*. 2006; 62(4):259-65.
27. Morris RGM. Developments of a water maze produced for studying spatial learning in the rats. *J Neurosci Meth*.1984; 11: 47-60.
28. Haley TJ, McCormick WG. Pharmacological effects produced by intracerebral injection of drugs in the conscious mice. *Br J Pharmacol*. 1957; b: 12-15.
29. Allain CC, Pool LS, Chan CS, G Richmond W, Paul CF. Enzymetic determination of total serum cholesterol. *Clin Chem*. 1974; 20: 470-75.
30. Stewart CA, Morris RGM. The water maze. In : Behavioural Neuroscience : A Pratical Approach. A. Sahgal. and P. Kimer (eds.). *Oxford University Press*, New York. 1993; pp. 107-22.
31. Packard MG, Teather LA, Bazan NG. Effect of intra-striated injections of platelet-activating factor and PAF antagonist BN 52021 on memory. *Neurobiol Learn Mem*. 1996; 66: 176-82.
32. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL. Inflammationn and Alzheimer's disease. *Neurobiol Aging*. 2000; 21: 383-21.
33. Etcheberrigary R, Ito E, Kim CS, Alkon DL. Soluble β-amyloid induction of Alzheimer's phenotype for human fibroblast K^+ channels. *Science*. 1994; 264: 276-79.
34. Chan C, Dharmarajan A, Atwood C, Huang X, Tanzi R, Bush A. Anti-apoptotic action of Alzheimer beta amyloid. *Alzheimer's Reports*. 1999; 2: 113-19.
35. Greenwood CE, Winocur G. Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty-acid intake. *Behav Neurosci*. 1996; 110: 451-59.
36. Greenwood CE, Winocur G. Glucose treatment reduces memory deficits in young adult rats fed high-fat diets. *Neurobiol Learn Mem*. 2001; 75: 179-89.
37. Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla FA. High-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience*. 2002; 112: 803-14.
38. Lannert H, Hoyer S. Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. *Behav Neurosci*. 1998; 112: 1199-08.
39. Sharma M, Gupta YK. Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats. *Pharmacol Biochem Behav*. 2001; 70: 325– 31.
40. Shoham S, Bejar C, Kovalev E, Weinstock M. Intracerebroventricular injection of streptozotocin causes neurotoxicity to myelin that contributes to spatial memory deficits in rats. *Exp Neurol*. 2003; 184: 1043– 52.
41. Mayer G, Nitsch R, Hoyer S. Effects of changes in peripheral and cerebral glucose metabolism on locomotor activity, learning and memory in adult male rats. *Brain Res*. 1990; 532: 95– 00.
42. Feillet-Coudray C, Rock E, Coudray C, Grzelkowska K, Azais-Braesco V, Dardevet D, Mazur A. Lipid peroxidation and antioxidant status in experimental diabetes. *Clin Chim Acta*. 1999; 284: 31– 43.
43. Reagan LP, Magarinos AM, Yee DK, Swzeda LI, Van Bueren A, McCall AL, McEwen BS. Oxidative stress and HNE conjugation of GLUT3 are increased in the hippocampus of diabetic rats subjected to stress. *Brain Res*. 2000; 862:292– 00.
44. Smith KJ, Kapoor R, Felts PA. Demyelination: the role of reactive oxygen and nitrogen species. *Brain Pathol*. 1999; 9: 69–92.
45. Braak H. Del Tredici K, Schultz C, Braak E. Vulnerability of select neuronal types to Alzheimer's disease. *Ann. N Y Acad Sci*. 2000; 924: 53–61.
46. Hoyer S, Muller D, Plaschke K. Desensitization of brain insulin receptor: effect on glucose/energy and related metabolism. *J Neural Transmission*. 1994; 44:(Suppl). 259-68.
47. Hoyer S. The aging brain. Changes in the neuronal insulin/insulin receptor signal transduction cascade trigger late onset sporadic Alzheimer's disease (SAD). A mini review. *J Neuronal Transmission*. 2000a; 109: 991-02.
48. Hoyer S. The brain insulin signal transduction system and sporadic (type II) Alzheimer's disease: an update. *J Neural Transmission*. 2000b; 109: 341-60 (Suppl).
49. Sharma B, Singh N, Singh M. Modulation of celecoxib and streptozotocin induced experimental dementia of Alzheimer's type by pitavastatin and donepezil. *J Psychopharmacol*. 2007 (in press)
50. Vaughan CJ, Delanty N. Neuroprotective properties of statins in cerebral ischemia and stroke. *Stroke*. 1999; 30: 1969-73.
51. Endres M, Laufa U. Effects of statins on endothelium and signaling mechanisms. *Stroke*. 2004; 35: 2708-11.
52. Amarenco P, Moskowitz MA. The dynamics of statins: from event prevention to neuroprotection. *Stroke*. 2006; 37: 294-96.

CURRENT AUTHOR ADDRESSES

Atish Prakash, Pharmacology Division, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala (Punjab), India. PIN: 147002.

Nirmal Singh, Pharmacology Division, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala (Punjab), India. PIN: 147002. E-mail; nirmal_puru@rediffmail.com (Corresponding author)

Manjeet Singh Pharmacology Division, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala (Punjab), India. PIN: 147002.