Research Article

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CORRELATION BETWEEN PROLONGED HYPERLIPIDEMIA & COGNITION IN WISTAR MALE RATS

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Abstract: The present study was undertaken to study relation between hyperlipidemia and memory loss and to assess protective effect of hypolipidemic drugs on memory, in rats. Hyperlipidemia was induced by feeding rats with the cholesterol-rich high fat diet for two months. At the end of study the parameters assessing memory were checked by three models, i.e. Passive Avoidance Test (Step In Latency-SIL), Elevated Plus Maze (Transfer Latency-TL) and Hebb Williams Maze (Time to reach Reward Chamber-TRC) followed by assessment of lipid profile. High fat diet groups showed memory impairment in all three models compared to normal diet groups and atorvastatin treatment with high fat diet showed significant protection from not only hyperlipidemia but also from memory loss in Hebb Williams maze and in Passive avoidance test. The present study indicates the advantage of controlling hyperlipidemia, which in turn is shown to protect from memory impairment.

Keywords: Hyperlipidemia, Cognition, High fat diet, Statins

INTRODUCTION

Dementia is defined as an acquired deterioration in cognitive abilities that impairs the successful performance of activities of daily living. Memory is the usual cognitive ability lost in dementia [1]. Alzheimer's disease (AD) is the cause of dementia in more than fifty percent of demented patients. The metabolic cardiovascular syndrome which includes hypertension, obesity, dyslipidemia, and glucose intolerance may be a preclinical condition which increases the risk of dementia [2]. However, elevated concentrations of plasma cholesterol are an important risk factor for cardiovascular disease, and now it is being suggested that metabolism of cholesterol plays role in the pathogenesis of AD [3].

Wolozin and colleagues undertook a study to determine whether patients on statins, have a lower prevalence of probable AD. This study demonstrated the association of statin therapy with a 60% to 73% lower prevalence rate of AD. Dufouil et al., showed that lipid-lowering agents are associated with decreased risk of dementia and hyperlipidemia is associated with increased risk of non-Alzheimer type dementia [4].

Controlled trials suggest that early diagnosis and treatment is very important in preventing dementia stage of Alzheimer's disease. Management of hyperlipidemia may be more effective in dementia and AD prevention [5].

The statistical association between use of LLA & low prevalence of vascular and Alzheimer's dementia presented by many observational human studies is though strong and statistically significant, cannot yet be considered a causal one as they used a cross-sectional analysis method, and also there is a likelihood of confounding by indication of statin therapy ("indication bias"). Therefore, there is need of studies to assess and establish role of hyperlipidemia & statins or control of hyperlipidemia in cognitive function. The present study was undertaken to study the effects of hyperlipidemia on memory and to assess protective effect of atorvastatin on memory loss, in wistar rats.

MATERIALS AND METHODS

Experimental groups:

Male wistar rats aged one month were divided in four groups [ND-Normal diet], ND+ATR -Atorvastatin], HFD - High fat diet] and [HFD+ATR] of eight animals each- [Table1]. They were housed in clean transparent poly propylene cages at room temperature with natural light – dark cycle. Animals in ND and ND+ATR group were fed on normal rat chow diet whereas HFD and HFD+ATR groups were given high fat diet for 60 days. Groups, ND+ATR and HFD+ATR were administered atorvastatin 10 mg/kg daily.

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Method of inducing hyperlipidemia:

Preparation of the cholesterol-rich high-fat diet: Deoxycholic acid (5g) was mixed with 700g of powdered rat chow diet. To this mixture was added cholesterol (5 g) dissolved in 300 g warm coconut oil. This cholesterol-rich high-fat diet (HFD) was molded into pellets of about 3 g each and was used to feed the animals [6].

Blood sample:

Blood sample obtained by cardiac puncture of anaesthetized rats was analyzed in the laboratory for complete blood lipid profile, after 60 days of starting the treatment [7]. Weight of the animals was recorded at the beginning and again, at the end of the study.

Learning and memory tests:

These tests were done on all four groups of animals after two months by following methods:

Passive Avoidance test:

Passive avoidance apparatus consists of a wooden box with a larger bright compartment and a smaller, dark compartment with grid floor which is attached to an electric shock source. On the first day of test rats were allowed to explore both chambers for five minutes. This was followed by three test trials of five minute each. In each trial, fraction of time spent in each compartment was measured. In fourth trial, as soon as rat stepped into dark compartment, a foot shock (2.5mA) was given and rat was replaced to home cage. After 24 hours, rats were placed in the test chamber and latency to enter the dark compartment was measured. Decreased latency to enter the dark compartment will suggest poor memory retention [8].

Elevated Plus Maze:

On the first day, each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the rat to move into any one of the covered arms with all its four legs. TL was recorded on the first day. If the rat did not enter into one of the covered arms within 90 seconds, it was gently pushed into one of the two covered arms and the TL was assigned as 90 seconds. The rat was allowed to explore the maze for additional 10 seconds and then is returned to its home cage. Memory retention is examined 24 h after the first day trial on the second day [9, 10].

Hebb Williams Maze:

It consists of three components: animal chamber (Start Box), which is attached to the middle chamber (Exploratory area) and a reward chamber at the other end of the maze in which the reward (Food) is kept. All the chambers are provided with guillotine removable doors. Twelve hours fasted rats were employed in the study. Each rat was placed in animal chamber (Start Box) and door was opened to facilitate the entry of the animal into the next chamber. The door of start box was closed immediately after the animal moved into the next chamber to prevent its back entry. Time taken (in seconds) by the rat to reach reward chamber (Time to reach Reward Chamber-TRC) from start box was noted for each animal. Each rat was allowed to explore the maze for additional 20 seconds, with all the doors opened before returning to its home cage. A fall in TRC on subsequent maze exposures was taken as an index of successful retention [11, 12]

Statistical analysis:

SPSS 17.0 software was used for analysis of data and nonparametric tests. Kruskal Wallis H test was used for comparison between the groups followed by Mann Whinteny U test for comparison between two individual groups.

Ethics: The study was approved by the IAEC.

RESULTS

Table-1: Effect of hyperlipidemia on weight gain and Learning-memory parameters [Mean±SEM]

Parameters	Normal Diet (ND)	Normal Diet with Atorvastatin (ND+ ATR)	High fat diet (HFD)	High fat diet with Atorvastatin (HFD+ ATR)
Total Cholesterol [mg/dl]	102.12 ± 2.61	*90.62 ± 3.68	168.12 ± 36.16	**106.50 ± 2.56
Triglyceride [mg/dl]	92 ± 4.50	74.87 ± 3.59	128.25 ± 25.38	**88.62 ± 3.59
HDL [mg/dl]	26.75 ± 2.73	*40.87 ± 3.83	35.00 ± 4.27	**48.37 ±
LDL [mg/dl]	56.97 ± 4.92	*34.85 ± 4.52	107.47 ± 27.31	**40.40 ± 2.87
Weight gain [Grams]	204.88 ± 9.12	190.25 ± 7.53	#266.00 ± 7.42	#250.63 ± 10.40
Passive avoidance(SIL) [Seconds]	207.12 ± 45.73	215.12 ± 38.40	57.62 ± 19.11	^191.25 ± 39.14
Elevated Plus Maze(TL) [Seconds]	32.37 ± 4.59	50.50 ± 8.96	64.75 ± 8.88	51.12 ± 7.29
Hebb-Williams Maze (TRC) [Seconds]	33.87 ± 4.63	21.38 ± 3.06	115.12 ± 29.64	^33.00 ± 5.18

n=8, *p<0.05 compared to ND, **p<0.05 compared to HFD, #p<0.05 compared to ND, ^p<0.05, compared to HFD+ATR

Table.1 shows the distribution of lipid profile, weight gain and the values of various tests conducted to evaluate learning and memory, at the end of study period.

Weight gain:

Weight gain was significantly higher in HFD group (266.00 ± 7.42 gms) and HFD+ATR (250.63 ± 10.40 gms) compared to ND group (204.88 ± 9.12 gms, $p<0.05$) while ND+ ATR group did not show significant change compared to ND group. There is no significant difference in weight gain of HFD group (66.00 ± 7.42 gms) compared to HFD+ ATR group (204.88 ± 9.12 gms, $p >0.05$)

Lipid profile:

There was marked reduction of TCh, TG and LDL levels and significant increase in HDL levels in ND+ATR treated group compared to ND group**.** Treatment with high fat diet showed marked increase in TCh, LDL and TG while no significant change in HDL compared to normal diet**.** Also there was marked increase in HDL in HFD+ ATR group compared to ND group, but didn't show significant difference in TG and TCh in comparison to HFD group. HFD + ATR group showed significant decrease in TCh, TG and LDL and increase in HDL level compared to HFD group.

Passive avoidancce performace

During passive avoidance exploration test, behaviour of rats of all groups, was not distinguishable. All the groups of rats spent similar time in the dark and bright compartments of passive avoidance appartus (data not shown). Latency to enter the dark compartment,: during passive avoidance retention test, HFD rats took shorter time to enter the dark compartment than the rats of other groups. Increase in the time taken to enter the dark compartment in HFD+ATR rats when compared to rats in HFD group was statistically significant. The increase in time among rats in ND+ATR group compared to rats on normal diet [ND] was not significant. [Table 1]

Elevated plus maze test:

The results summarized in Table 1 show that there is no significat difference in transfer latency [TL] among rats in ND group compared to ND+ATR group or HFD group and HFD+ATR group.

Hebb-Williams Maze:

Time taken by animal (learning score) to reach the reward chamber from the entry chamber was significantly decreased in HFD + ATR groups compared to HFD group. The difference in time to reach reward chamber of ND+ATR group and ND group was not statistically significant.

DISCUSSION

Hyperlipidemia was induced in rats by feeding them on high fat diet for 60 days. Total cholesterol, triglycerides, and LDL level significantly increased in HFD group compared to ND group. There were no significant differences in HDL level in ND and HFD groups. Atorvastatin as expected protected animals against hyperlipidemia. Beneficial effect of Atorvastatin was also observed in ND group. Atorvastatin did not have any significant effect on weight gain.

Three models, as discussed above, were used to assess memory of animals in various groups. In passive avoidance test, rats in HFD group, entered the dark compartment early, which suggests failure to retain memory of painful stimulus i.e. footpad shock. Atorvastatin protected from such memory impairment in hypolipidemic rats fed on high fat diet [HFD]. In elevated plus maze improvement in memory was not demonstrated in any of the two groups [ND+ATR and HFD+ATR]. In Hebb Williams's maze, rats of HFD+ATR group compared HFD group took less time to reach reward chamber from start chamber which indicates retention of learned task (memory).

HFD groups showed memory impairment in all three models compared to normal diet groups and atorvastatin treatment of rats fed on high fat diet [HFD+ATR] showed significant protection from not only hyperlipidemia but also from memory loss in Hebb Williams maze and in Passive avoidance test.

When effect on memory of atorvastatin treated group on normal diet [ND+ATR] was compared with animals on normal diet without atorvastatin [ND], there was not significant change of memory retention in in any of the experimental models which suggests that atorvastatin treatment in the absence of any significant hyperlipidemia may not protect from memory loss to a great extent. Memory protection was apparent and significant when effects are compared among rats having hyperlipidemia [HFD] to rats with hyperlipidemia treated with atorvastatin [HFD+ATR], indicating lowering of elevated lipid levels may be the major factor for memory protective effect of statins.

Though atorvastatin lowered lipid levels in ND rats, this effect did not result in improvement of memory status in ND+ATR rats. However memory was greatly impaired in HFD rats compared to rats in ND group. Improvement in memory status was obvious when these rats [HFD] were treated with atorvastatin. Hence it can be suggested that atorvastatin is more effective in improving memory when it is adversely affected as a result of increased lipid levels

CONCLUSION

The results of this study suggest that controlling hyperlipidemia also in turns protects from memory impairment. This can establish hyperlipidemia as one of the causative factor in development of dementia and further broaden the use of lipid lowering agents.

However, further studies involving neurochemical assays and histological examination of brain tissue, are necessary to explore the possible pathophysiology of memory loss due to hyperlipidemia and mechanism of protection from cognitive impairment by lipid lowering agents.

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