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Atorvastatin improves Y-maze learning behaviour in nicotine treated male albino rats

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ABSTRACT

Nicotine is a parasympathomimetic alkaloid present in tobacco which can induce hyperlipidemia and has a direct effect on neural functions. Statins, competitive inhibitors of 3-hydroxymethyl-3-glutaryl-coenzyme-A reductase, are cholesterol lowering drugs. It has some neuroprotective effects. Hence we analysed the combined effect of nicotine and statin on the learning behaviour of male albino rats. We employed Y-Maze conditional discrimination task. Rats were divided into 4 groups with six rats in each group. (1) Control, (2) Atorvastatin (10 mg/kg b.wt), (3) Nicotine (0.6 mg/kg b.wt) and (4) Atorvastatin (10 mg/kg b.wt) + Nicotine (0.6 mg/kg b.wt). After 30 days of treatment rats from each group were selected for behavioural study and they were observed for 30 days. At the end of the experimental period rats were sacrificed, and brain and liver were dissected out for further biochemical analysis. Nicotine treated group showed least performance in learning in comparison with control, atorvastatin and atorvastatin + nicotine treated groups. Co-administration of atorvastatin and nicotine improved learning behaviour compared to nicotine treated group. Reactive oxygen species level was significantly increased in nicotine group compared to control. The level of neurotransmitter serotonin which has a significant role in learning was found to be decreased in nicotine treated group compared to the control group. Activity of Na^+ K^+ ATPase, Ca^{2+} ATPase and glutathione content was significantly reduced in nicotine treated group compared to control. The activity of acetylcholine esterase was significantly increased in the nicotine treated group. Expression studies showed significant decrease in N-methyl D-aspartate receptors and increase in mono amine oxidase-A and mono amine oxidase-B in nicotine treated group and was reversed in atorvastatin + nicotine treated group. It can be concluded that co-administration of nicotine with statin ameliorates the neural functional alterations caused by nicotine to a significant level.

1. Introduction

Cognitive functions, especially learning behaviour is necessary for the survival of an organism and it depends on healthy neurons and normal neurotransmitter levels; the intercellular messengers of nervous system. Nicotine, the root cause of tobacco addiction is well known for its effects on nervous system (Middlekauff et al., 2014). According to the WHO statistical report on tobacco epidemic approximately 5000 million deaths occurred in global population due to tobacco generated diseases (WHO, 2008). It can induce reactive oxygen species (ROS) production by the activation of NADPH oxidase (Mahapatra et al., 2009). Nicotine plays its role by mimicking biological neurotransmitter acetylcholine and thereby activating nicotinic acetylcholine receptor (nAchR) to stimulate dopamine release. Dopamine plays a key role in reward system and forms the basis of nicotine addiction (Benowitz, 2008) and is able to down regulate N-methyl D-aspartate (NMDA) receptors which are involved in learning behaviour (Salamone et al., 2014).

NMDA receptor can be considered as one of the fundamental neurotransmitter receptors in brain which shows a high affinity for glutamate (Sulkowski et al., 2014). Binding of glutamate activates NMDA receptors which causes high Ca^{2+} influx. This influx is thought to be responsible for strengthening of synapses through long term potentiation (LTP) and weakening through long-term depression (LTD) (Zito et al., 2009). Overstimulation of NMDA receptors results in neuronal excitotoxicity (Tu et al., 2014) by a variety of aberrant transcriptional cascades and redox mediated post translational modifications. Nicotine also induces hyperlipidemia (Gossett et al., 2009).

Atorvastatin is a lipophilic HMG CoA reductase inhibitor widely used for the treatment of hypercholesterolemia (Izawa et al., 2015). It possess pleiotropic effects (Sarath et al., 2014) such as antioxidant effect by inhibiting NADPH oxidase (Najah et al., 2008) and anti-inflammatory effect by indirectly activating cytochrome oxidase-2 (COX-2) whose products are prostaglandins and thromboxanes (Puccetti et al., 2011;

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Xiao et al., 2013). Atorvastatin shows significant effect on cognitive functions in rats with diazepam-induced amnesia (Georgieva-Kotetarova and Kostadinova, 2013).

Nicotine and atorvastatin possess neuromodulatory effects. So we focused our study on the combined effect of nicotine and atorvastatin on learning behaviour of male rats in a Y-maze conditional discrimination task.

2. Materials and methods

Male albino rats (Sprague Dawley strain, average wt of 175 ± 25 g) were selected and housed in polypropylene cages, kept in a room at 28°–32 °C. The light cycle was 12 h light and 12 h dark. Animals were handled as per the laboratory animal welfare guidelines.

2.1. Groups

Animals were divided into four groups (6 animals in each group) as follows:

Group (C) Control fed with normal diet
Group (A) Atorvastatin (10 mg/kg body wt/day).
Group (N) Nicotine (0.6 mg/kg body wt/day).
Group (A + N) Atorvastatin + Nicotine (10 mg/kg body wt/day + 0.6 mg/kg body wt/day).

Atorvastatin (Sigma-Aldrich, St. Louis, MO, USA) was freshly dissolved in distilled water and given orally by gastric tube. Nicotine was injected intraperitoneally. The dose of the nicotine was selected from previous studies (Seema et al., 2007). Rats were fed with standard laboratory diet supplied by Ashirwad Pvt. Ltd., India and water was given ad libitum. The duration of the experiment was 60 days. After 30 days of treatment rats from each group were selected for behavioural study and they were observed for 30 days. At the end of the experimental period rats were anaesthetised with chloral hydrate. Their whole brain and liver were immediately dissected out, cleaned with ice-cold saline, blotted dry and transferred to ice-cold containers for various biochemical analysis. Animal experiments were approved by the Institutional Animal Ethics Committee [IAEC No-KU-25/2011-BC-MI (31)].

2.2. Y-maze learning test

Learning ability was tested, as described by Murray and Ridley (1997), in a wooden Y-maze, which had three arms of equal size (60 cm long, 11.5 cm wide and 25 cm height). The arm where the rats were placed at the beginning of each trial was considered the start arm. The other arms, had food cups located at the ends and they were considered the choice arms. The experiment consisted of placing the rats at the start arm and letting them move to the correct arm holding food pellets. Pre-training was carried out to familiarize the rats with the maze. The whole area of the choice arms of the maze was covered by black or white inserts (59 cm long, 11 cm wide and 25 cm height). To assess the cognitive behaviour, black and white inserts were alternatively used in the choice arms. In each trial, rats were rewarded for choosing the left arm when the inserts were black and the right arm when the inserts were white. The reward for the correct response consisted of four food pellets placed in the food cup at the end of the correct arm. If the rat made an incorrect response, it was allowed to go to the empty food cup at the end of the incorrect arm and was removed after 5 s. Proper training was given to all rats prior to the experiment. During experiment six trials were given to each rat and the time for each trial ended when the rat chose the correct arm or a maximum of 10 min.

Performance index was calculated by the formula.

$$\text{Performance index} = \frac{\text{number of correct turns–number of incorrect turns.}}{\text{Time taken in minutes}}$$

2.3. Biochemical assays

Activity of HMG CoA reductase was determined in liver homogenate by the method of Rao and Ramakrishnan (1975). All other biochemical indices were measured in whole brain homogenate as follows: Mitochondrial ROS level was measured by the method of Driver et al. (2000). The activity of acetylcholine esterase was estimated by the method of Ellmann and Courtney (1961). Tissue samples for the estimation of neurotransmitters were prepared according to the procedure of Persky and Reese (1971). Activity of sodium potassium ATPase (Na^+ K^+ ATPase) and calcium ATPase (Ca^{2+} ATP ase) were assayed by the method of Brunberg and Halmi (1966).

2.4. Quantification and expressions of NMDA-NR1, monoamine oxidase-A(MAO-A) and monoamine oxidase-B (MAO-B) mRNAs

Total RNA was isolated from brain using TRI reagent (Sigma Aldrich) by the method described by Chomcynski and Sacchi (1987). The isolated RNA was used for Reverse Transcriptase-Polymerase Chain reaction (RTPCR) to quantify the expression of NMDA-NR1, MAO-A and MAO-B genes. Total RNA was reverse transcribed and PCR was performed using Eppendorf RT-PCR kit with gene specific primers. Sequences of the primers are given in Table 1. PCR mixture was resolved on 2% agarose gel containing ethidium bromide. Then the gels were subjected to densitometric scanning (Bio-Rad Gel Doc, California, USA) to determine the optical density of each and then normalized against an internal control, β-actin using Quantity One imaging software.

2.5. Statistical analysis

Statistical analyses were carried out using the Statistical Package for Social Science.

(SPSS Inc. Chicago, IL, USA) version 17.0. Data generated in behaviour study was analysed with Paired sample t-Test to analyse day to day difference and with normal distribution and homogenous variance were analysed using one-way analysis of variance (ANOVA). Pair fed comparisons between the groups was made by Duncan's multiple range tests. $p \leq 0.05$ was considered to be significant.

3. Results

3.1. Y-maze conditional discrimination task

The results showed (Fig. 1) that atorvastatin treated group showed a slight reduction in performance compared to control. And nicotine administered group showed poor performance index compared to control

Table 1
Primer Sequences.

Primers	Sequence	Gene accession No.
β-Actin	Forward 5′-ACCCGCGAGTACAACCTTCT-3′ Reverse 5′-ATGGCTACGTACATGGCTGG-3′	NM_031144.3
NMDAR-NR1	Forward 5′-GGAGTCCAAGGCAGAGAAGG-3′ Reverse 5′ -CTCCTGTGTGCCAAACTTGC-3′	NM_017010.2
MAO-A	Forward 5′ –AAGACACGCTCAGGAATGGG-3′ Reverse 5′ –TCAAAATCGGTGGGATGGCA-3′	NM_033653.1
MAO-B	Forward 5′ –TTTGGCAGCCAGAACCAGAA-3′ Reverse 5′ –AGCTTGTGTTCCAGTCACCC-3′	NM_013198.1