

LONG TERM ADMINISTRATION OF HMG-COA-REDUCTASE INHIBITOR (SIMVASTATIN) AFFECTS BRAIN SEROTONIN NEUROTRANSMISSION IN MALE RATS

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Abstract

Simvastatin, an important member of statin family is widely prescribed as cholesterol-lowering agent. Like other statins it acts by inhibiting the rate limiting enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, responsible for the endogenous production of cholesterol which forms an essential part of neuronal cell membranes. Lowering of cholesterol has been reported to alter the brain chemistry and hence neurotransmission. To understand the association between low cholesterol and brain serotonin (5-HT) we monitored the effect of oral administration of simvastatin for 4 weeks on brain serotonin levels. Drug treated rats exhibited significantly low plasma cholesterol levels. Brain serotonin and 5-HIAA (5-hydroxyindole acetic acid) levels were also decreased in drug treated rats. Plasma tryptophan (TRP) was significantly increased but brain tryptophan levels were significantly decreased in drug treated rats. Weekly food intake during the entire experimental period was comparable in control and drug treated rats. Results of the present study suggest that simvastatin induced lowering of cholesterol may be responsible for the decrease in brain 5-HT neurotransmission and hence may be a cause of depression observed in subjects taking simvastatin to lower cholesterol levels.

Keywords: Simvastatin; plasma cholesterol; TRP; brain serotonin; brain 5-HIAA.

INTRODUCTION

Cholesterol plays an important role in the development, function and stability of synapses. Evidence shows that low cholesterol influences 5-HT function (Branchey *et al.*, 2000). Studies also show that subjects having low cholesterol exhibit suicidal, agitated and criminal behavior (Golomb *et al.*, 2000). These altered behaviours have been attributed to the reduced number of serotonin receptors in brain as a result of low cholesterol in membranes (Kaplan *et al.*, 1997, a). A positive correlation between the incidence of dementias, Alzheimer's disease and plasma cholesterol levels has been shown earlier (Evans *et al.*, 2000). Animals kept on a low-cholesterol diet were more aggressive and exhibited lower CSF concentrations of 5-hydroxyindoleacetic acid (5-HIAA) compared to their high-cholesterol counterparts (Kaplan *et al.*, 1994). Lowering of serum cholesterol may alter the membrane microviscosity and hence affect the serotonin neurotransmission. Serotonin pathways function as a behavioural system that inhibits impulsive behaviour. Low cholesterol levels could thus facilitate such behaviours as violence towards the self or others (Vevera *et al.*, 2005). The relationship among dietary cholesterol, serotonergic activity, and social behaviour is also reported from other species and experiments. Evidence shows that dietary lipids can influence brain neurochemistry and behaviour; this phenomenon could be relevant to the understanding of the increase in suicide and violence-

related death observed in cholesterol-lowering trials (Kaplan *et al.*, 1994).

The low Plasma cholesterol affects the cholesterol homeostasis in the brain. It has been shown that the blood brain barrier (BBB) is permeable to molecules such as sterols. Hydroxylation of sterols increases the rate of diffusion through the BBB (Dietschy and Turley, 2004). Plasma cholesterol levels correlate with brain 27-OH cholesterol levels which maintains cholesterol homeostasis in the brain (Bjorkhem *et al.*, 2006; Björkhem, 2006; Björkhem *et al.*, 1998). Hydroxylated cholesterol has been shown to passively cross the BBB (Dietschy and Turley, 2004; Cornford *et al.*, 1982). The principle carrier protein in the brain is ApoE which is the major apolipoprotein in plasma. Studies have shown that low levels of ApoE increase behavioural deficits, oxidative stress and synaptic dysfunction (Lauderback *et al.*, 2001; Zhou *et al.*, 1998).

A role of 5-HT has been implicated in various psychological disorders. Chemical hypothesis of depression suggests that these disorders are caused by lowered concentration of serotonin in the brain which is treated by antidepressant drugs (Blair and Wards, 2003). Evidence shows that changes in membrane fluidity alter the serotonin neurotransmission (Engelberg, 1992; Scanlon *et al.*, 2001). Synthesis of cholesterol in the brain could be altered by cholesterol-lowering drugs that can

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cross the BBB. Simvastatin being a lipophilic statin penetrates the BBB (Saheki *et al.*, 1996). It has been shown that administration of simvastatin decreases the levels of cholesterol in the brain which affects the cholesterol distribution (Kirsch *et al.*, 2003) and leads to a decrease in cholesterol turnover in the brain (Locatelli *et al.*, 2002).

The aim of present study was to investigate the role of simvastatin in the neurochemical deficits produced by its chronic use in rats. The study was also aimed to determine the association between lowered cholesterol levels and brain 5-HT metabolism.

MATERIALS AND METHODS

Animals

Twenty adult male Wistar rats weighing 180-200gm purchased from the Aga Khan Medical University were used in the study. All animals were housed individually under a 12 h light/ 12h dark cycle (lights on at 06:00h) and controlled room temperature ($22 \pm 2^\circ\text{C}$) with free access to cubes of standard rodent diet for at least 5 days before experimentation. All experiments were carried out according to the guidelines approved by Local Animal Care Ethical Committee.

Experimental Procedure

Animals were randomly divided into control and drug treated groups. Each group composed of 10 rats. Control rats were given tap water orally while the rats in test group were given a high dose of Simvastatin 100 mg/kg (Karin *et al.*, 2005) orally, daily for 4 weeks.

Sample Collection

After 4 weeks of treatment the rats were decapitated. Blood was collected for plasma cholesterol and plasma Tryptophan estimations. Brain samples were excised very quickly from the cranial cavity within 30 seconds of the decapitation. Fresh brains were dipped in chilled saline and stored at low temperature (-70°C) until analysis of 5HT and 5HIAA by HPLC-EC. The experiments were performed in a balanced design in such a way that the control and test rats were killed alternatively to avoid the order effect.

Neurochemical Estimations

HPLC-EC determination was carried out as standard (Haleem and Haider, 1996). A 5-II Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer at pH 2.9, on Shimadzu LEC 6A detector at an operating potential of 0.8 volts for biogenic amines and 1.0 volts for tryptophan.

RESULTS

Figure 1 shows the effect of simvastatin administration on plasma cholesterol levels. Data analyzed by Student's *t*-test revealed a significant decline ($p < 0.01$) in cholesterol levels after 4 week of drug administration.

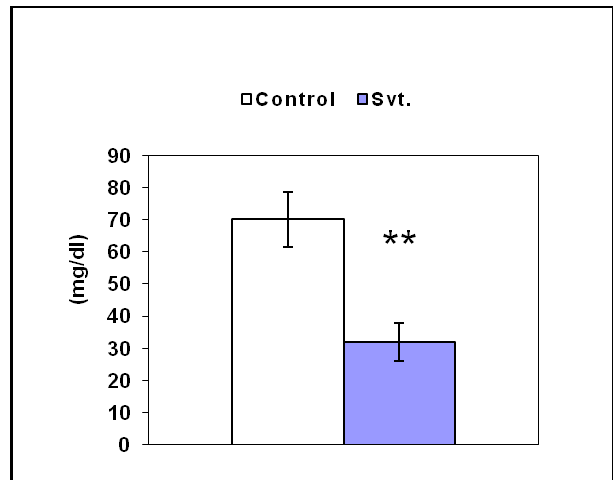


Fig. 1. Effect of Simvastatin (Svt.) administration on plasma cholesterol levels in rats. Values are mean \pm S.D (n=10). Significant difference by Student's *t*-test; ** $p < 0.01$ vs control rats.

Figure 2 shows the effect of simvastatin administration on plasma tryptophan levels. Data analyzed by Student's *t*-test showed a significant increase ($p < 0.05$) in plasma tryptophan levels.

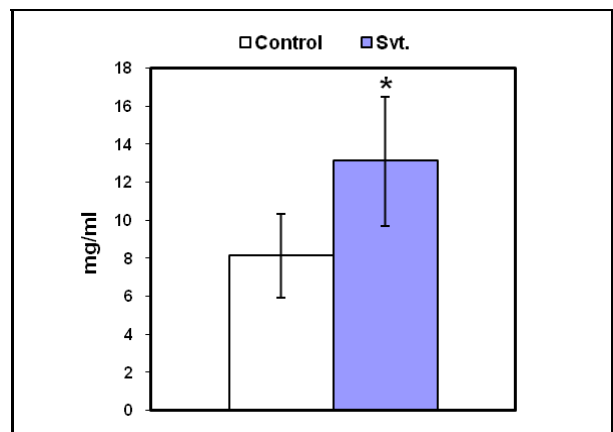


Fig. 2. Effect of Simvastatin administration on plasma Tryptophan levels in rats. Values are mean \pm S.D (n=10). Significant difference by Student's *t*-test; * $p < 0.05$ vs control rats.

Figure 3 shows the effect of simvastatin administration on brain tryptophan levels. Analysis by Student's *t*-test revealed a significant decrease ($p < 0.05$) in brain tryptophan levels.

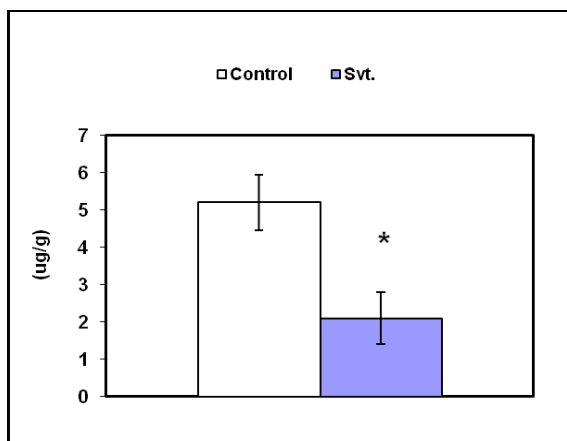


Fig. 3. Effect of Simvastatin administration on brain Tryptophan levels in rats. Values are mean \pm S.D (n=10). Significant difference by Student's *t*-test; *p<0.05 vs control rats

Figure 4a and b show the effect of simvastatin administration on brain 5-HT and 5-HIAA levels in the brain. Data analyzed by Student's *t*-test revealed a significant decrease (p<0.05) in both 5-HT and 5-HIAA levels.

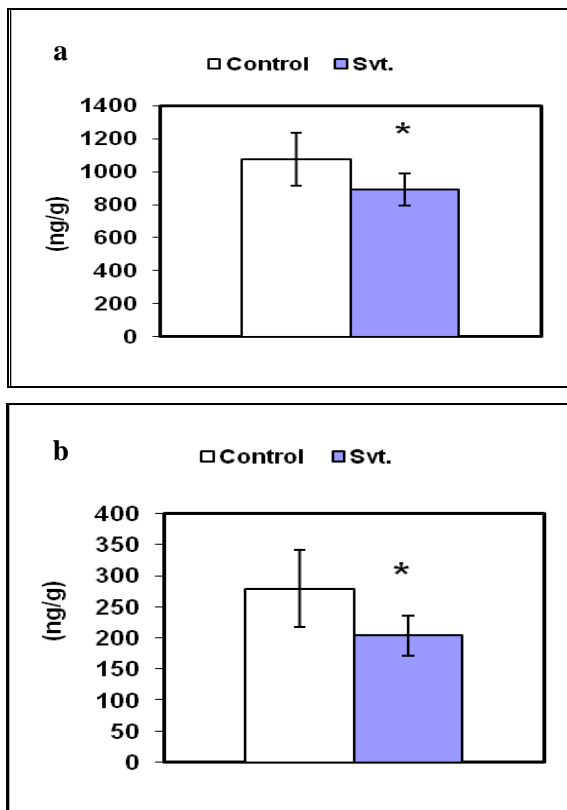


Figure 4: Effect of Simvastatin administration on Brain 5-HT (a) and 5-HIAA (b) levels in rats. Values are mean \pm S.D (n=10). Significant difference by Student's *t*-test; *p<0.05 vs control rats

Figure 5 shows the effect of simvastatin administration on weekly food intake. Data analyzed by Student's *t*-test revealed no significant difference in the food intake during the experimental period.

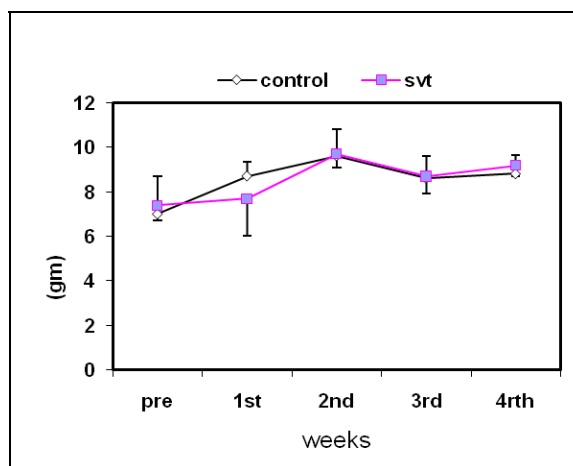


Fig. 5. Effect of Simvastatin administration on weekly food intake in rats. Values are mean \pm S.D (n=10). Student's *t*-test showed no significant difference

Statistical Analysis

The statistical significance of the results was computed by Student's *t*-test; p value < 0.05 were considered as significant.

DISCUSSION

The present study is concerned with the effects of low cholesterol on brain 5-HT neurotransmission. The results showed that the daily administration of Simvastatin (100 mg/kg) for 4 weeks produced a significant decline in plasma total cholesterol levels as compared to controls. A 38 % decrease in cholesterol compared to controls was observed following drug administration. Brain 5-HT and 5-HIAA levels were also significantly decreased in drug treated rats. Food intake during 4 week was comparable between both groups. Drug treated rats exhibited a significant increase in plasma TRP and a significant decrease in brain TRP levels. The decreased uptake of brain TRP in absence of a decrease in plasma TRP may be explainable in terms of an increase in plasma fraction of bound TRP. HMG Co-A reductase inhibitor has been shown to lower plasma free fatty acid (FFA) level (William *et al.*, 2006). Decreased plasma cholesterol levels have also been associated with a decrease in plasma FFA (Branchey and Branchey, 2003). The decrease in brain level of TRP may be due to a decrease in plasma free fraction of TRP as more TRP is present in bound form with albumin, thus decreasing the uptake of TRP from plasma to brain. In the present study plasma levels of FFA were not measured however the increase in

plasma TRP levels in drug treated rats suggest that a decrease in FFA make the albumin more available for TRP hence increasing its bound form in plasma. The rate of 5-HT synthesis is totally dependent upon the brain concentration of its precursor TRP (Curzon and Murphy, 1986). Therefore decreased brain 5-HT and its metabolite 5-HIAA in present study may be attributed to the decrease in brain TRP levels. The decrease in brain 5-HT levels may also be explained by the direct action of lipophilic simvastatin which crosses the BBB (Saheki *et al.*, 1996). Evidence shows that administration of simvastatin decreases the cholesterol concentration in synaptosomal membrane and affects cholesterol distribution (Kirsch *et al.*, 2003), which may alter the 5-HT neurotransmission.

In the present study a high dose of simvastatin was used which produced a decrease in brain 5-HT metabolism. It has been suggested earlier that central serotonin neurotransmission may be affected by the altered microviscosity of plasma membrane due to an altered cholesterol level (Engelberg, 1992; Terao *et al.*, 2000). Evidence shows that low cholesterol influences 5-HT metabolism in brain (Branchey *et al.*, 2000). Evidence also shows that the brain lipids are vulnerable to variations in serum lipids (Geiser, 1990; Garber *et al.*, 1996). Circulating complex lipoprotein particles carry triglycerides and phospholipids as well as fat soluble micronutrients to the brain. So the decrease of brain serotonin level in the present study following 4 week administration of HMG CoA reductase inhibitor could be due to the altered serum lipids. Indeed the explanation of cholesterol exchange between central and peripheral pool has been challenged by some authors but others support the concept (Cibickova *et al.*, 2008). The present study shows decreased 5-HIAA levels following the simvastatin administration. Evidence shows that animals on a low cholesterol diet were more aggressive and exhibited lower CSF 5-HIAA levels than animals with higher cholesterol (Kaplan *et al.*, 1997, b). There is also evidence that low levels of cholesterol could affect membrane fluidity (Block and Edwards, 1987).

CONCLUSION

In the light of above findings we may conclude that, the reduced levels of 5-HT and its metabolite 5-HIAA in the present study could be due to the lowered levels of cholesterol that affect the membrane fluidity, leading to altered 5-HT metabolism. Moreover lack of any effect on food intake in drug treated animal's shows that the decrease in brain tryptophan levels is not a side effect of the drug but may be due to the decreased levels of plasma free fraction of tryptophan. The decrease in brain 5-HT neurotransmission following simvastatin administration may be the cause of depression reported in subjects taking simvastatin to lower cholesterol levels.

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