ORIGINAL ARTICLE

EFFECTS OF SIMVASTATIN ON LIPID PROFILE AND NERVE CONDUCTION VELOCITY IN OBESE SPRAGUE DAWLEY RATS

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Background: The incidence of obesity is increasing worldwide. The lipid derangements and decrease in nerve conduction velocity are important complications for which a number of treatment options are being considered. In this study, Simvastatin, a hydroxyl methyl glutaryl coenzyme A reductase inhibitor is studied for its effects on these complications of obesity. **Methods:** The study was a randomised control trial conducted at Islamic International Medical College, Rawalpindi in collaboration with Railway General Hospital, Rawalpindi, and National Institute of Health, Islamabad. Ninety adult male Sprague Dawley rats were divided into three groups with thirty rats in each group. One group of rats was taken as control with normal diet while other two groups were given High Fat Diet (HFD) for the whole study period, i.e., 10 weeks. One of the HFD group was given Simvastatin along with high fat diet for four weeks. Lipid profile was done by enzymatic colorimetric method. Conduction velocity of sciatic nerve was determined with the help of PowerLab® data acquisition system. **Results:** The two groups with HFD showed more than 25% increase in weight at the end of study as compared to control group. HFD group showed significantly higher lipid profile and decreased sciatic nerve conduction velocity when compared with control. The group that was given Simvastatin showed significant improvement in lipid profile and increased sciatic nerve conduction velocity after 4 weeks when compared with the group that was given HFD without any intervention. **Conclusions:** Simvastatin is effective for improving the lipid profile and sciatic nerve conduction velocity in HFD induced obesity.

Keywords: Obesity, Simvastatin, nerve conduction velocity

INTRODUCTION

The obesity is a serious public health as well as economic problem.[1] The prevalence of obesity has risen by three folds or more in many countries since 1980. It is estimated that globally there are about 1.6 billion overweight adults; at least 400 millions are obese.[2] The prevalence of obesity among Pakistani population is 28% while the incidence among the people more than thirty years of age is 47%.[3] Rapid industrialisation and change in life styles with minimal physical activity are important contributing factors to its development.[4]

Genetic, metabolic, behavioural and environmental factors are among the causes of obesity.[5] The rapid increase in the incidence of obesity is related with increased energy intake and physical inactivity, both of which are influenced by socioeconomic status and environmental factors.[3] Dietary fat is considered to be one of the most important environmental factor in development of obesity, therefore high fat diet induced obesity may serve as effective model to investigate the pathophysiological mechanisms that may lead to complications.[6]

The obesity is associated with a number of health consequences including deranged lipid profile. The dyslipidemia that occurs in obesity leads to complications including cardiovascular diseases with atherosclerosis, neuropathy, cerebrovascular accidents and respiratory problems.[7]

Lipid abnormalities related to obesity include an elevated serum cholesterol, low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol and triglycerides.[8] There is also a reduction in serum high density lipoprotein cholesterol. The cause of this dyslipidemia is increased hepatic triglyceride synthesis from lipolysis of an increased adipose tissue.[9]

In obese subjects, there is an increased risk of developing carpal tunnel syndrome, sensory and motor nerve conduction abnormalities.

There is also decreased amplitude of compound action potentials of tibial, peroneal and ulnar nerves in obese as compared to the lean individuals.[10] The toxic oxygen radicals, decreased endoneurial blood flow or accumulation of lipids around the nerves may cause this neuropathy.[11]

Among the treatment strategies for obesity and its complications, there is a role of lipid lowering drugs like statins, bile acid sequestrants, fibrates and cholesterol absorption inhibitors.[12] There can be a possible role of antioxidants because literature supports the involvement of the free oxygen radicals in the development of neuropathy in obesity.[11]

Simvastatin was the 1st statin that was used extensively in clinical practice. It inhibits the enzyme 3-hydroxy, 3-methyle glutaryle Co-A reductase, thus inhibiting the first committed step of sterol synthesis. Another action is to upregulate the LDL-C receptors along with the anti-inflammatory effect.[13] Its role for improving the decreased conduction velocity of nerves in obesity was not found in literature. Therefore this study

was planned to evaluate the role of Simvastatin in ameliorating the neuropathy of obesity.

MATERIAL AND METHODS

The study was a randomised control trial, done at Islamic International Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad, and Railway General Hospital, Rawalpindi from December 2008 to November 2009.

Ninety male, adult Sprague Dawley rats weighing 200±25 gm were kept in animal house of NIH. Animals were placed in cages of 2×3 feet size, ten animals per cage. Temperature was maintained at 24±2 ºC along with twelve hour light and dark cycle. Rats were subjected to an adaptation period of one week in which the food intake and body weight was monitored before any intervention according to the experimental plan. Rats were then followed throughout the study for adequate intake of food and water and weight gain by weighing them weekly.

Rats were divided into three groups with 30 rats in each group (n=30). Group I served as a control and was given the normal standard rat diet. The rats were injected with the vehicle (distilled water) through the subcutaneous route daily during the study. Group II was fed with a high fat diet for ten weeks with no intervention. High fat diet contained 230 gm/Kg diet of butter fat in addition to the standard contents of rat diet.[14] The rats were injected with subcutaneous vehicle (distilled water) throughout the study. Group III was fed with a high fat diet for first six weeks and then given subcutaneous Simvastatin in the dose of 5 mg/Kg/day for next four weeks.[13] High fat diet was continued along with the drug.

Diseased rat or any rat that developed disease during the course of study was excluded from the study. Study Variables were lipid profile and sciatic nerve conduction velocity. Lipid profile included the serum total cholesterol, serum triglycerides, serum low density lipoproteins and serum high density lipoproteins. At the end of 10 weeks of study, 2 cc blood was taken from each rat by intracardiac blood sampling. The lipid profile was done at The Railway General Hospital, Rawalpindi by enzymatic colorimetric method (ENDPOINT). Cromatest kits (Spain) were used that were run on Merck Analyzer, Microlab-200. Sciatic nerve was dissected from each rat and conduction velocity of sciatic nerve was calculated with the help of PowerLab® data acquisition system. Statistical analysis was done with the help of SPSS-16. Mean values and standard deviations were calculated for all the data. Confidence interval was taken as 95%. For multiple comparisons, a one way analysis of variance (ANOVA) was used and $p<0.05$ was taken as significant.

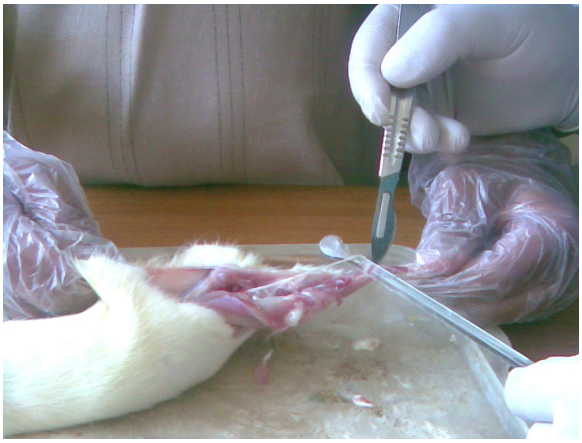

Figure-1: Sciatic nerve dissection showing the sciatic nerve over the glass rod

RESULTS

All rats remained alive, healthy and active throughout the period of study. The initial weight of all the rats was noted on day one of the study. They were monitored throughout for increase in weight and final weight was noted at the end of study. On the same day their lipid profile was done along with the determination of sciatic nerve conduction velocity. In the end of study, the mean weight of control with normal diet was 214.57 gm with increase of 24%. The mean weight of control with high fat diet (HFD) was 301.1 gm with 75% increase in weight. The weights of group III (HFD and Simvastatin) was 289.63 gm and increase in weight of 68%. Table-1 shows significant decrease in the levels of serum total cholesterol, triglyceride and low density lipoprotein levels in the group of rats that was given Simvastatin during the study. The comparison showed highly significant difference (p=0.001) with lowest value of LDL in group III with Simvastatin supplementation. Table-2 shows the improvement in sciatic nerve conduction velocity by Simvastatin supplementation in group III as compared to the decrease in conduction velocity in group II by HFD.

Table-1: Effects of Simvastatin on levels of serum total cholesterol, triglyceride, HDL and LDL deranged by HFD in obese rats (n=30)

Lipid profile (Serum) (mg/dl)	Control (n=30)	HFD (n=30)	HFD+ Simvastatin (n=30)	p*
Total Cholesterol	24.4±10.32	89.6±25.86	51.43±16.66	0.001
TG	62.97±28.09	160.67±48.80	125.77±53.81	0.004
HDL	12.93±6.32	19.40±7.79	21.17±8.63	0.001
LDL	6.73±1.98	37.90±15.41	14.57±12.19	0.000

*$p<0.05$ is taken as significant

Table-2: Effect of Simvastatin on sciatic nerve conduction velocity deranged by HFD in obese rats

Control (m/s) Mean±SD	HFD (m/s) Mean±SD	HFD+Simvastatin (m/s) Mean±SD	p*
88.67±5.35	30.50±8.36	61.93±13.30	0.002

*$p<0.01$ is taken as significant

DISCUSSION

The obesity and its complications are well recognised worldwide. This study was planned to see the treatment option for the obese individuals who are suffering from neuropathy with decreased conduction velocity of nerves. As the Simvastatin which belongs to HMG-CoA reductase inhibitor class of lipid lowering drugs is effective for dyslipidemia, its role is studied for improving the sciatic nerve conduction velocity.

In the present study, ninety days old male Sprague Dawley rats after 10 weeks of high fat diet, gained more than 25% of weight compared to control with normal diet. This weight gain was similar to the study done by Levin and Dunn-Meynell in which HFD was given to Sprague Dawley rats for 10 weeks.[15]

The control group of rats that was given high fat diet in this study showed a significant increase in the lipid profile at the end of study as compared to the control with normal diet. There were increased levels of serum total cholesterol, serum triglycerides and low density lipoproteins while the high density lipoprotein levels were lowered when compared with control group. The results were similar to the HFD induced increase in total cholesterol and serum triglycerides in the study done by Jeon and Kim[14] in which HFD was given for a period of six weeks.

Four week Simvastatin, as proved by Tawfik *et al*, in the same dose as our study, significantly lowered the values of serum total cholesterol and serum triglycerides.[13] The increase in serum total cholesterol, triglyceride and low density lipoprotein levels were similar in the study conducted by Penumathsa *et al*, in which male Sprague Dawley rats were fed 2% cholesterol diet for eight weeks.[16] Lipid lowering effects of statins were similar to our study with the remarkable decrease in TC, TG and LDL levels. HDL levels are also improved remarkably with Simvastatin along with the reduction of TG and LDL.[17]

In the study conducted by Adameova *et al*, male Wistar rats were given Simvastatin at the dose of 10 mg/Kg as a component of normal and high cholesterol diet for 5 days.[18] There, Simvastatin did not improve the altered lipid levels that may be due to the oral route of administration for short duration of 5 days. While in our study, the drug was given by the subcutaneous route for 4 weeks. Dose dependant effects of Simvastatin were proved by Collins *et al*, by the study that showed marked improvement of LDL levels with high dose of Simvastatin.[19]

The relationship of obesity and nerve conduction deficits was observed in a study conducted by Miscio *et al*, in which the non-diabetic obese individuals even without symptoms suggestive of peripheral neuropathy were studied for nerve conduction deficits.[10] The obese group showed significantly decreased compound muscle action potential amplitude of tibial and peroneal nerves and decreased sensory action potential amplitude of all nerves. This showed a subclinical impairment of nerve conduction in obese individuals. It is reported that HFD fed female mice after sixteen weeks developed obesity along with motor and sensory nerve conduction deficits.[20] This deficit in nerve conduction is similar to our study in which impaired nerve conduction developed after 10 weeks of HFD. So far in the literature, Simvastatin, a lipid lowering drug used in our study has not been found for being studied directly as a treatment option for peripheral neuropathy but in a study conducted by Sabri *et al* on mice, it was proved that Simvastatin markedly reduces the oxidative stress that is a contributory factor for neuropathy.[21]

This could be a better treatment option for obese individuals who are having deranged lipid profile along with the impaired nerve conduction velocity that presents with numbness and tingling sensations in the limbs. The results of this study after four week treatment with Simvastatin showed significant improvement of sciatic nerve conduction velocity of rats as compared to the control group who received HFD throughout the study period.

Based on findings of present study, it is suggested that in obesity, there is a decrease in sciatic nerve conduction velocity. Simvastatin improves altered lipid profile but also contributes to improvement of nerve conduction velocity in obesity.

CONCLUSIONS

HFD induced obesity resulted in decrease in sciatic nerve conduction velocity. Simvastatin was effective for improving the deranged lipid levels and sciatic nerve conduction velocity was also improved in obese rats.

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