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EFFECT OF CHRONIC ADMINISTRATION OF ATORVASTATIN, SIMVASTATIN AND LOVASTATIN ON ANIMAL MODELS OF EPILEPSY

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ABSTRACT

Introduction: Statins, the widely used hypolipidemics drugs have a number of pleotropic effects.
Objective: To study the effect of chronic administration of atorvastatin, simvastatin and lovastatin on maximal electroshock and pentylenetetrazole induced seizures in Wistar rats.
Material and Methods: After obtaining institutional animal ethics committee clearance the study was conducted in male wistar rats. The animals were dosed with the various statins for 30 days. The effect of statins on maximal electroshock and pentylenetetrazole induced seizures was then studied. The animals were then sacrificed and the brain tissue was used for antioxidant estimation.
Results: Statins showed a protective effect against seizures in both the models. The levels of glutathione were increased and malonadialdehyde decreased in the brain tissue in statin treated groups. The present study confirms the anticonvulsant action of statins, their antioxidant action being the possible mechanism.
Keywords: statins, seizures , antioxidant action

INTRODUCTION

Statins, the most widely used and efficacious hypolipidemic which act by inhibiting HMG-CoA reductase enzyme are now known to have many pleotropic effects[1] .The pleiotropic effects of statins include improving endothelial function, enhancing stability of plaques, decreasing oxidative stress, inflammation and inhibiting thrombosis[1,2].

It has been hypothesized that statins reduce the risk of developing epilepsy in the elderly. [3] High dose atorvastatin has been shown to reduce the frequency of tonic-clonic seizures induced by quinolinic or kainic acid. [4] A cohort study also showed that statins reduced the hospitalization due to seizures[3.] Based on these studies, the authors had studied the effect of statins in animal models of epilepsy after a single dosing, wherein simvastatin had reduced the duration of seizures in maximum electroshock (MES) model without abolishing hindlimb extension. In pentylenetetrazole (PTZ) model simvastatin and lovastatin decreased the duration of seizures and also increased the latency for the onset of seizures in comparison to the control group. Atorvastatin at a dose of 3.6mg/kg increased the latency but had no effect on duration of seizures in PTZ model. [5] The results of the study indicated that statins do have an antiepileptic action but needed further confirmation. Hence the authors planned to study the effect of chronic administration of various statins on MES and PTZ induced seizures and also their impact on oxidative changes which occur during seizures.

MATERIALS AND METHODS

Animals

Albino rats weighing 150-200g were used for the study. They were maintained under standard conditions in Central animal house, Manipal University, Manipal approved by the CPCSEA.

The rats were kept in polypropylene cages (U.N. Shah Manufacturers, Mumbai) and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro industries Ltd, Sangli, Maharashtra) and water ad libitum. The rats were maintained at a room temperature 26 ±20C, relative humidity 45-55% and light/ dark cycle of 12 h.

Reagents : Special chemicals such as 5 5'-dithio-bis (2-nitrobenzoic acid) (DTNB), 1-cholro 2, 4-dinitrobenzene (CDNB) ,reduced glutathione (GSH), thiobarbituric acid (TBA), trichloroacetic acid (TCA) were obtained from Sigma Chemicals Co. (St Louis, MO). All the reagents used were of analytical grade

Drugs: Atorvastatin (Zydus Cadila Healthcare Ltd), simvastatin (Micro Labs Ltd), lovastatin (Dr.Reddy's Laboratories Ltd), carbamazepine (Novartis India Ltd, Mumbai), sodium valproate (Sun Pharmaceutical Industries Ltd, Mumbai) and pentylenetetrazole (Sigma – Aldrich,

Mumbai) were used for the study. The doses selected were in accordance to that of the previous study done by the authors. [5]

Methodology: The study was undertaken after obtaining permission from the Institutional Animal Ethics committee, Manipal. A total of sixty animals were used for the study. They were divided into two sets for testing in two experimental models, the maximal electroshock and pentylenetetrazole seizures.

I: Maximal electroshock model

Rats were divided into 5 groups (n=6). The groups I to V received gum acacia (1ml), carbamazepine (108mg/kg), atorvastatin (7.2 mg/kg), simvastatin (1.80mg/kg) and lovastatin (3.60mg/kg) respectively orally once daily for 30 days. Maximal electroshock seizures was induced as described by Toman et al[6] with a current of 150 mA delivered through the ear clip electrode for 0.2 sec with the help of convulsiometer after a month of drug administration. Absence of hind limb extension (HLE) was taken as protection against seizures. Only the animals which show hind limb extension during the screening procedure on the earlier day were included in the study.

II: PTZ induced seizures[7]

Rats were divided into 5 groups (n=6).The groups I to V received gum acacia (1ml),sodium valproate (180mg/kg), atorvastatin (7.2mg/kg), simvastatin (1.80mg/kg) and lovastatin (3.60mg/kg) respectively orally once daily for 30 days. Pentylenetetrazole (60mg/kg i.p) was then given to induce seizures after a month of drug administration. Each animal was then placed in an individual cage for observation lasting 30min. The duration of the seizures in each group was recorded.

BIOCHEMICAL ESTIMATIONS

Collection of tissue samples: The animals were sacrificed by cervical dislocation and brain tissues were carefully removed and chilled in ice-cold phosphate buffer. After washing in ice-cold buffer, the hippocampus was dissected out and homogenized in phosphate buffer (pH 7.4, 10% w/v) using Teflon homogenizer. The tissue homogenate was then utilized for

malondialdehyde (MDA) assay and glutathione-S-transferase (GST) assay.

Malondialdehyde assay Level of MDA was analyzed in the rat brain by the method of Okhawa *et al* with slight modifications[8].100µL of homogenate, 1000µL of TBA and 500 µL of TCA were mixed and incubated at 100° for 20 minutes , then centrifuged at 12000 rpm for 5minutes . Absorbance was read spectrophotometrically at 532 nm.

Glutathione-S-transferase assay GST assay was analyzed in the rat brain by the method of Habig.[9] Phosphate buffer (850µL), CDNB (50 µL) and GSH (50 µL) were mixed and incubated at 37°C for ten minutes, then 50 µLof homogenate was

added and absorbance was read spectrophotometrically at 340 nm.

Statistics: The results were analysed by one way ANOVA followed by Dunnett's test. Statistical analysis was done using the SPSS 16.0 version.

RESULTS

In the present study, there was a significant ($p \leq 0.05$) reduction in the duration of PTZ induced seizures in atorvastatin, lovastatin, simvastatin and sodium valproate treated groups in comparison to the control group (62.93±3.43). In maximal electroshock induced seizure model carbamazepine, atorvastatin, simvastatin and lovastatin significantly (< 0.01) reduced the duration of seizures in comparison to control group (Table1). The tissue glutathione levels were significantly ($p<0.05$) increased in the atorvastatin and simvastatin treated groups and malanodialdehyde levels were significantly ($p<0.05$) reduced by all the treated groups in comparison to control (Table 2).

DISCUSSION

The present study demonstrates the anticonvulsant action of statins following chronic administration. In the previous study conducted by the authors, atorvastatin following a single dose had not shown antiseizure activity in both the models. Hence in the present study the dose of atorvastatin had been increased. The higher dose of atorvastatin reduced the duration of seizures both in the MES and PTZ models. Our findings are in accordance to the earlier study reports[5]. The protective effect of higher dose of atorvastatin against seizures can be attributed to its poor lipophilicity.

Neuronal hyper excitability and oxidative stress have been suggested to be responsible for various neurological diseases and epilepsy. Studies have shown that antioxidants can reduce seizure manifestations and the markers of oxidative stress [10]. Oxidative stress is one of the etiological factors in inducing seizures in various epilepsy models. Drugs with antioxidant action have a potential as adjuvants to antiepileptic drugs. [11]

Peroxidation of polyunsaturated fatty acids in cell membrane produces malonadialdehyde. The production of MDA is used as a marker to measure the level of oxidative damage in tissues. The nervous tissue is prone for damage by oxidative stress due to large amounts of polyunsaturated fatty acids which are susceptible for lipid peroxidation. Elevated levels of MDA in the brain issue have been detected in patients with epilepsy.[12] In the present study atorvastatin , simvastatin and lovastatin significantly decreased the MDA levels in the brain when compared to control group. Hence, the statins by virtue of their antioxidant action reduced the levels of elevated MDA produced due to oxidative stress.

Cellular glutathione which is a major antioxidant is maintained by enzymes such as glutathione-s – transferase and glutathione reductase and the inhibition of activities of these enzymes therefore would deplete cellular reduced glutathione level and increase the accumulation of toxic metabolites [13]. In the present study the statins replenished the stores of glutathione which was depleted in the control group, suggesting their antioxidant action. Production of nitric oxide, [14] inhibition of neuroinflammation[15] have also been suggested to be the possible mechanism by which statins can produce antiseizure activity.

CONCLUSION

In the present study, statins showed an anticonvulsant action. Antioxidant action of statins as the possible mechanism of their anticonvulsant action can also be hypothesized from this study. Further research and clinical trials are warranted to justify their use either alone or as adjuvants to antiepileptics in future.

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REFERENCES

1. Bersot TP. Drug therapy for hypercholesterolemia and dyslipidemia. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman's The pharmacological basis of therapeutics .12thed. New York: Mc Graw Hill ; 2006.p. 877-908.
2. Zhou Q, Liao JK. Pleiotropic effects of statins. Circulation journal. 2010; 74: 818-826
3. Das RR, Herman ST. Statins in epilepsy. Neurology 2010; 75: 1490-1491
4. Lee JK Won JS, Singh AK, Singh I. Statin inhibits kainic acid-induced seizure and associated inflammation and hippocampal cell death. Neurosci Lett 2008; 440(3):260-4.
5. Nayak V, Adiga S, Poornima BM, Sharma R, Garg A. Effect of atorvastatin, simvastatin,lovastatin on animal models of epilepsy : a comparative study. International journal of current research and review 2012; 4(11):30-33.
6. Toman JEP, Swinyard EA, Goodman LS. Properties of maximal seizures and their alteration by anticonvulsant drugs and other agents. J Neurophysiol 1946; 9:231-39.
7. Visweswari G, Prasad KS, Chetan PS, Lokanatha V, Rajendra W. Evaluation of the anticonvulsant effect of *Centella asiatica* (gotu kola) in pentylenetetrazol-induced seizures with respect to cholinergic neurotransmission. Epilepsy and Behavior 2010; 17(3):332-335.
8. Okhawa H, Ohishi N, Yagi K. Anal Biochem 1979; 95**:**351–358.
9. Habig WH, Pabst MJ, Jakaby WB. Glutathione-S-transferase: the first enzymatic tep in mercapturic acid formation. J Biol Chem 1974;29**:** 7130-7139.
10. Devi PU, Manocha A, Vohora D. Seizures, antiepileptics,antioxidantsand oxidative stress :an insight for researchers. Expert Opin Pharmacother 2008; 9(18):3169-77.
11. Shin EJ, Jeong JH, Chung YH, Kim WK, Ko KH, Bach JH, et al. Role of oxidative stress in epileptic seizures. Neurochem Int 2011;59(2):122-37.
12. Pandey MK, Mittra P, Maheshwari PK. The lipid peroxidation product as a marker of oxidative stress in epilepsy. JCDR 2012; 6(4): 590-92.
13. Mueller SG, Trabesinger AH, Boesiger P, Wieser HG. Brain glutathione levels in patients with epilepsy measured by in vivo(1)H-MRS. Neurology 2001; 57(8): 1422-47.
14. Moazzami K, Emamzadeh-Fard S, Shabani M. Anticonvulsive effect of atorvastatin on pentylenetetrazole induced seizures in mice: the role of nitric oxide pathway. Fundamental and clinical pharmacology 2013; 27:387-92.
15. Mirhadi K. Anticonvulsant effect of pitavastatin in mice induced by pentylenetetrazole. American journal of animal and veterinary sciences 2011; 6(4):166-70.

Table 1: Duration of seizures in PTZ and MES model

Groups	Duration of seizures in PTZ model (secs) (Mean±SEM)	Groups	Duration of seizures in MES model (secs) (Mean±SEM)
Control	62.93±3.43	Control	31.88±1.63
Sodium valproate	49.33±2.20*	Carbamazepine	14.49±0.31*
Atorvastatin	16.5±0.84*	Atorvastatin	15.03±0.5*
Simvastatin	44.17±6.95*	Simvastatin	13.12±0.91*
Lovastatin	48.00±4.95*	Lovastatin	17.5±0.25*

*p≤ 0.05 in comparison to control

Table 2: Antioxidant levels in groups treated with statins

Groups	Glutathione/g of tissue	Malonaldehyde ml/g of tissue
Control	5.95±0.74	275.73±22.05
Atorvastatin	25.67±1.84**	110.36±3.98**
Simvastatin	32.80±4.6**	140.02±4.55**
Lovastatin	4.75±1.51	106.57±9.09**

**p≤ 0.01 in comparison to control