



Effect of vestibular stimulation on lipid profile in MPTP induced Parkinsonian mouse model

Harini Narayanam¹, Archana Rajagopalan^{2*}, Subramani Parasuraman³, Suresh V Chinni⁴

¹Department of Physiology, Faculty of Medicine, Manipal University College Malaysia, Melaka, Malaysia,

²Department of Physiology, Saveetha Medical College, Saveetha Institute of Technical and Medical Sciences (SIMATS), Chennai, India,

³Department of Pharmacology, Faculty of Pharmacy, AIMST University, Kedah, Malaysia,

⁴Department of Biochemistry, Faculty of Medicine, Bioscience, and Nursing, MAHSA University, Selangor, Malaysia

*Correspondence: Dr Archana Rajagopalan
Department of Physiology, Saveetha Medical College

Abstract

The changes in blood lipid levels in Parkinson's disease (PD) patients and their clinical significance remains unclear till date. Many studies have found that lipid metabolic abnormalities may be a major cause of Parkinson's disease (PD). The present study was undertaken to study the effect of vestibular stimulation on body weight and lipid profile in MPTP induced mice. Total of 24 male mice are divided into 4 groups with 6 mice in each group by simple random sampling. Mice were given intraperitoneal injection of MPTP at a dose of 30mg/kg body weight once daily for 5 consecutive days to induce PD. Caloric vestibular stimulation was applied by instilling warm (40 °C) water into external ear of the mice. At the end of the intervention mice were sacrificed and blood was collected by cardiac puncture. Total cholesterol, triglycerides, HDL, and LDL were analysed in all the groups. Our study shows that caloric vestibular stimulation has a positive effect on the levels of total cholesterol, triglycerides, high density lipoprotein, and low-density lipoprotein. Further studies with a large sample size are needed to establish vestibular stimulation as a new therapeutic agent in treatment of PD.

Keywords : Lipid profile, body weight, MPTP, Parkinson's

DOI Number: 10.14704/nq.2022.20.8.NQ44362

NeuroQuantology 2022; 20(8): 3327-3331

Introduction

Parkinson disease (PD) is a prevalent neurological disorder and has a prevalence of roughly 2% in adults aged 65 and older. Clinical signs of Parkinson's disease include

motor symptoms, such as motor delay, resting tremor, and muscle rigidity, cognitive dysfunction is the most prevalent nonmotor symptom., which includes mild cognitive impairment and dementia. Patients and their



family are adversely affected by the onset and progression of PD dyskinesia and cognitive dysfunction; therefore, it is of utmost importance to prevent and delay these conditions in aged patients (Xue Hong). Recent research indicates that lipids and uric acid levels in the blood may be associated with the incidence of PD (M. X. Dong). It has been hypothesised that cholesterol and/or its oxidised metabolites (oxysterols) may have a possible endogenous responsibility in the development of Parkinson disease. Clinical investigations point to a direct relationship between increased levels of cholesterol in plasma and the prevalence of PD. Additionally, a high-fat diet worsens Parkinsonian pathologies in animal models of the disease, including the loss of dopamine-neurons (Paul R). In some recent animal studies, it was discovered that vestibular stimulation can successfully control TG and total cholesterol, assisting in the maintenance of a healthy body weight (Saritha S). Vestibular stimulation promotes sleep and regulates food intake via the vagus nerve, insulin, arcuate nucleus, thyroid hormones, and PA-axis. (Sadanandan NN).

In the present study Caloric, vestibular stimulation (CVS) is used to stimulate the vestibular apparatus. It is a simple and non-invasive procedure that is shown to improve the motor and non-motor symptoms of PD in pre-clinical experiments (Jagadeesan T). The primary goal of this research is to determine the impact of bilateral CVS on lipid profile in MPTP induced Swiss albino mice.

Materials and Methods

Experimental animals

After receiving ethical approval from the AIMST University Animal Ethics Committee (AUAEC/FOM/2020/04), 24 healthy male Swiss albino mice weighing 25-30 gm were used for the study. Following the minimal number of animals used in previous studies and to obtain statistically significant results, the sample size of 6 animals per group was chosen. The animals were kept under a 12-hour light/dark cycle with temperature and humidity controls in spacious polyacrylic

cages. Food and water were available to the animals' ad libitum.

MPTP animal model

MPTP was purchased from Sigma Aldrich, USA, and dissolved in sterile saline. Mice were given intraperitoneal injection of MPTP at a dose of 30mg/kg body weight once daily for 5 consecutive days to induce dopaminergic neuron death in the substantia nigra. (Zhu YL et al, 2019, Schober, 2004)

Caloric vestibular stimulation

Hot water at a temperature of 42°C, was used to irrigate the middle ear canals of the mice. 2 ml of hot water was delivered into each ear with a polyethylene tube. The flow rate was kept at 0.2ml/s to ensure continual stimulation of the vestibular system. (Sadanandan S).

Experimental design

Swiss albino mice were divided into four groups at random. Each group had 6 animals.

Group 1: Normal control

Group 2: MPTP control

Group 3: Only CVS for 30 days

Group 4: MPTP + CVS for 30 days

Body weight of all the mice was estimated on 1st, 7th, 14th, 21st and 28th day.

Blood Collection

Mice were anaesthetized with chloral hydrate (350 mg/kg; i.p.) at the end of the experiment, and blood was collected via cardiac puncture. The serum was separated by centrifugation at 3000 x g for 4 minutes and used for analysis (Paul R)

Lipid Profile Analysis

The serum total cholesterol was estimated by in vitro enzymatic technique using a colorimetric kit CHOD-PAP method (Allain CC) as per the manufacturer's instructions. The GPO-PAP method was used to calculate triglycerides (Henkel). The precipitation method was used to estimate HDL and LDL. (Demacker, Kerscher).

Data analysis

The findings were tabulated as Mean \pm SEM. For statistical analysis, the one-way ANOVA was used, followed by Tukey's post hoc test. Statistical analyses were performed by GraphPad prism 8.0 software.

Results



Body weight

Although there was a slight decrease in the weight of mice treated with MPTP when compared to that of the normal control, it was

not statistically significant as given in table 1. There was no difference between the control and CVS groups.

Group	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control	27.40 ± 0.71	27.72 ± 0.64	28.02 ± 0.70	28.17 ± 0.59	28.23 ± 0.68
MPTP control	26.68 ± 1.27	26.37 ± 1.26	25.68 ± 1.32	25.60 ± 1.28	25.25 ± 1.32
CVS 30 days	27.05 ± 0.70	27.38 ± 0.65	27.48 ± 0.65	27.82 ± 0.65	27.83 ± 0.68
MPTP+ CVS 30 days	28.08 ± 0.59	28.17 ± 0.53	28.23 ± 0.51	28.42 ± 0.54	28.52 ± 0.56

Table 1: Effect of caloric vestibular stimulation on MPTP treated mice in various Results are tabulated as mean ± SEM and P<0.05 was considered statistically significant. There is no statistical difference in the weight of the mice.

Effect of vestibular stimulation on Lipid Profile

Mice that were treated with MPTP showed an increase in the Total Cholesterol (TC), but the difference was not statistically significant as shown in Table 2. However, administering CVS has decreased the values as seen in the treatment group. No difference was seen in the group of mice that received only vestibular stimulation.

As shown in Table 2, there was a noticeable increase in the values of Triglycerides (TG) in the MPTP treated group when compared to the normal mice. CVS has reduced the values of TG in the treatment group, but the

difference is not statistically significant. There was no significant effect of CVS on TGs in the CVS alone group.

The study suggested that HDL level in the MPTP treated mice was markedly lower than that of healthy controls. CVS has significantly increased the values of HDL in both the MPTP treated mice and CVS alone groups (Table 2). Both LDL and vLDL levels were significantly increased in the MPTP treated mice in the current study. CVS has significantly increased the values of both LDL and vLDL in mice administered with MPTP and in CVS alone group (Table 2).

3329

Group	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	vLDL (mg/dL)	Atherogenic Index (AI)
Normal control	117.50 ± 3.85	80.83 ± 2.30	43.83 ± 1.92	20.17 ± 1.45	4.03 ± 0.29	0.27 ± 0.03
MPTP control	143.00 ± 10.40	113.33 ± 10.03**	24.17 ± 1.62***	30.50 ± 0.99**	6.10 ± 0.20**	0.67 ± 0.04***
CVS 30 days	115.83 ± 6.02	95.00 ± 5.03	42.00 ± 2.90###	22.67 ± 2.12#	4.53 ± 0.42#	0.36 ± 0.04###
MPTP + CVS 30 days	121.33 ± 6.04	95.17 ± 5.49	36.50 ± 2.17###	23.67 ± 1.93#	4.73 ± 0.39#	0.42 ± 0.04####

All the values are mean ± SEM (n = 6). **P<0.01 and ***P<0.001 compared with that of control; #P<0.05, ##P<0.01 and ###P<0.001 compared with that of PD control (One-way ANOVA followed by Tukey's *post-hoc* test).

Discussion

In the present study MPTP was administered to the mice to induce Parkinson disease (PD).

The serum total cholesterol level in the animal's post MPTP administration was slightly decreased and the values improved



post CVS intervention, however this change was not statistically significant. HDL was lowered and TG, LDL and vLDL values were increased in the animals that were given MPTP. The incidence of PD is second only to that of Alzheimer's disease among middle-aged and older individuals (Z song). Blood lipids have emerged as a biochemical blood index in recent years, that may be associated with Parkinson's disease in numerous clinical trials, but the results of these research are inconsistent (Garcia Sanz). Lipids can protect the nervous system. According to research HDL-C in lipids can actively contribute to the anti-inflammatory and antioxidant effects. The increased risk of neuronal degeneration is correlated with the decline in HDL-C levels (Hong X). The association of TC with PD is still controversial and inconclusive as some studies have shown that there is lower risk of PD with higher serum TC (Miyake Y) and some studies have shown otherwise (Hu). Lipid-lowering drugs are thought to offer therapeutic potential in PD because of their anti-inflammatory, antioxidant, and antiplatelet actions in animal or cell studies (Bar-On P). Numerous studies have also revealed that lipid-lowering medication use was somewhat beneficial in PD patients (Fu X). In our study, vestibular stimulation decreased the levels of serum TC, TG, LDL and vLDL and increased the levels of HDL. Neha et al in their study have showed that vestibular stimulation has significantly reduced the levels of serum TC, TG and LDL in premenstrual women (Sara MN). Antihyperlipidemic effect of vestibular stimulation was also proven by Neethu et al in their animal study (Sadanandan).

Conclusion

Vestibular stimulation was found to be effective in reducing the levels of serum cholesterol, Triglycerides and LDL and increased the levels of HDL. To investigate the relationship between lipid levels and PD severity or clinical progression, larger sample size studies are required. Effective intervention measures for cholesterol and its metabolites will have a significant clinical impact in delaying the onset of Parkinson's

disease, and vestibular stimulation may become a new therapeutic agent.

References

1. Allain CC, Poon LS, Chan CS, Richmond WF, Fu PC. Enzymatic determination of total serum cholesterol. *Clinical chemistry*. 1974 Apr 1;20(4):470-5.
2. Bar-On P, Crews L, Koob AO, Mizuno H, Adame A, Spencer B, Masliah E. Statins reduce neuronal α -synuclein aggregation in in vitro models of Parkinson's disease. *Journal of neurochemistry*. 2008 Jun;105(5):1656-67.
3. Demacker PN, Hessels M, Toenhake-Dijkstra H, Baadenhuijsen H. Precipitation methods for high-density lipoprotein cholesterol measurement compared, and final evaluation under routine operating conditions of a method with a low sample-to-reagent ratio. *Clin Chem*. 1997 Apr;43(4):663-8. PMID: 9105270.
4. Dong MX, Wei YD, Hu L. Lipid metabolic dysregulation is involved in Parkinson's disease dementia. *Metabolic Brain Disease*. 2021 Mar;36(3):463-70.
5. Fu X, Wang Y, He X, Li H, Liu H, Zhang X. A systematic review and meta-analysis of serum cholesterol and triglyceride levels in patients with Parkinson's disease. *Lipids in health and disease*. 2020 Dec;19(1):1-0.
6. García-Sanz P, MFG Aerts J, Moratalla R. The Role of Cholesterol in α -Synuclein and Lewy Body Pathology in GBA1 Parkinson's Disease. *Movement Disorders*. 2021 May;36(5):1070-85.
7. Henkel, E., Stoltz, M. A newly drafted colour test for the determination of triglycerides convenient for manual and mechanized analysis (glycerolphosphate-oxidase — PAP method). *Z. Anal. Chem.* **311**, 451–452 (1982). <https://doi.org/10.1007/BF00481817>
8. Hong X, Guo W, Li S. Lower Blood Lipid Level Is Associated with the Occurrence of Parkinson's Disease: A Meta-Analysis and Systematic Review. *International Journal of Clinical Practice*. 2022 Jun 9;2022.



9. Hu G, Antikainen R, Jousilahti P, Kivipelto M, Tuomilehto J. Total cholesterol and the risk of Parkinson disease. *Neurology*. 2008 May 20;70(21):1972-9.
10. Jagadeesan, T., Rajagopal, A. and Sivanesan, S., 2021. Vestibular stimulation: a noninvasive brain stimulation in Parkinson's disease & its implications. *Journal of Complementary and Integrative Medicine*, 18(4), pp.657-665
11. Kerscher L, Schiefer S, Draeger B, Maier J, Ziegenhorn J. Precipitation methods for the determination of LDL-cholesterol. *Clinical Biochemistry*. 1985 Apr 1;18(2):118-25.
12. Miyake Y, Tanaka K, Fukushima W, Sasaki S, Kiyohara C, Tsuboi Y, Yamada T, Oeda T, Miki T, Kawamura N, Sakae N. Case-control study of risk of Parkinson's disease in relation to hypertension, hypercholesterolemia, and diabetes in Japan. *Journal of the neurological sciences*. 2010 Jun 15;293(1-2):82-6.
13. Paul R, Choudhury A, Kumar S, Giri A, Sandhir R, Borah A (2017) Cholesterol contributes to dopamine-neuronal loss in MPTP mouse model of Parkinson's disease: Involvement of mitochondrial dysfunctions and oxidative stress *PLoS ONE* 12(2): e0171285. doi:10.1371
14. Sadanandan NN, Archana R, Kumar SS, Mukkadan JK, Antony NJ. Antihyperlipidemic effect of vestibular stimulation in wistar albino rats. *Int J Res Ayurveda Pharm*. 2015; 6:509-12.
15. Sara MN, Shalu MM. *INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH*.
16. Saritha S, Sailesh KS, Mukkadan JK. Impact of linear vestibular stimulation on lipid profile in underweight females: a pilot study. *J Med Sci Health*. 2017;3(3):43-6.
17. Schober, A., 2004. Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. *Cell and tissue research*, 318(1), pp.215-224.
18. Z. Song, S. Liu, X. Li et al., "Prevalence of Parkinson's disease in China: a multicenter population-based survey," *Neuroepidemiology*, vol. 56, 2021.
19. Zhu, Y.L., Sun, M.F., Jia, X.B., Cheng, K., Xu, Y.D., Zhou, Z.L., Zhang, P.H., Qiao, C.M., Cui, C., Chen, X. and Yang, X.S., 2019. Neuroprotective effects of Astilbin on MPTP-induced Parkinson's disease mice: Glial reaction, α -synuclein expression and oxidative stress. *International Immunopharmacology*, 66, pp.19-27.

