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An Investigation into Anti-Dyslipidemic Activity of Isovaleric Acid in Wistar Rats Fed Fructose-Rich High Fat Diet

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Abstract

Introduction: *Correction of dyslipidemiais important for prevention of atherosclerotic diseases. Limitations of the currently available anti-dyslipidemic drugs prompted us to investigate the anti-dyslipidemic activity of isovaleric acid isolated from Valeriana wallichi.*

Methods: *The study was conducted on 24 healthy male adult Wistarrats (200-225 gm) divided into four groups of six each. Group I was kept on normal diet (Hindustan Lever Food Pellets). Group II was fedfructose-rich high fat diet (F-HFD). Group III was given F-HFD+isovaleric acid (25 mg/kg of body weight/day), and group IV was given F-HFD+ atorvastatin (10 mg/kg body weight/day). After 30 days, plasma lipids and lipoproteins were measured in all the rats.*

Results: *Feeding F-HFD resulted in significant dyslipidemia. Isovaleric acid and atorvastatin were equally effective in preventing the F-HFD-induced dyslipidemia.*

Conclusions: *Further studies are warranted to realize the anti-dyslipidemic potential of isovaleric acid in animals and humans.*

Keywords: *Dyslipidemia; isovaleric acid; atorvastatin.*

Introduction

Dyslipidemia usually comprises an increase in serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C), and a decrease in high density lipoprotein cholesterol (HDL-C), either singly or in combination.[1] Long term dyslipidemia results in vascular changes and dysfunction.[2] Therefore, dyslipidemia is recognized as a risk factor in the initiation and development of atherosclerosis and cardiovascular diseases.[3,4] It has also been shown

that controlling dyslipidemia for long periods with the currently available anti-dyslipidemic drugs is not easy.[3,4,5]Therefore, there is a need to develop safe and effective anti-dyslipidemic agents.

Isovaleric acid is otherwise known as 3-methylbutanoic acid.[6]It is usually added as a flavoring agent to wine.[6]It has been proposed that it is an anticonvulsant.[7]Isovaleric acid is a component of *Valeriana wallichi*, a plant that we are investigating as a possible anti-dyslipidemic therapy in animals. In view of the encouraging results obtained so far (unpublished observations), we decided to investigate whether the anti-dyslipidemic activity of *Valeriana wallichi* might be due to isovaleric acid present in it. Atorvastatin was used as a reference drug for comparison.

Material and Methods

The study was conducted on24 male Wistar rats (200-225 gm) after receiving approval from the institutional ethics committee of King George's Medical University, Lucknow. The rats were divided into four groups of six each. Initially, all the groups were kept on Hindustan Lever Food Pellets and water ad libitum. They were put in cages and kept in a temperature and humidity controlled room with a 12-hour light-darkcycle. After acclimatization, group I (normal diet control) continued to receive normal pellet diet. Group II was switched to fructose-rich high fat diet (F-HFD) to induce dyslipidemia. F-HFD (1 kg) was prepared by mixing 610 gm of normal pellet diet, 280 gm of ground nut oil, 5 gm of cholesterol, 5 gm of deoxycholic acid and 100 gm of fructose. Group III was given F-HFD and oral isovaleric acid (25 mg/kg of body weight/day) isolated from *V. wallichi*. Group IV was given F-HFD and oral atorvastatin (10 mg/kg of body weight/day). At the end of experimental period (30 days), blood samples were collected from retro-orbital plexus of each rat in EDTA tubes, and plasma was separated by centrifugation.

Plasma cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C)and low density lipoprotein cholesterol (LDL-C) were measured by CHOD-PAP,GPO-POD, PEG-PAP and immunoturbidimetric methods.[8,9,10,11,12] Very low density lipoprotein cholesterol (VLDL-C) was calculated by Friedewald formula.[13] Estimation of free fatty acids (FFA) was done by the method of Monsinger et al.[14] Phospholipids in plasma were determined by Malachite green method.[15] Plasma lecithin cholesterol acyltransferase (LCAT) activity was measured by the method of Albers et al.[16] Wind and Robinson method was used to measure post heparin lipolytic activity (PHLA).[17]

Statistical Analysis

InStat3 package was used for statistical analysis. The mean± SD of different groups were compared by using one way analysis of variance (ANOVA), followed by Student-Newman-Keuls test. $P<0.05$ was considered significant.

Results

Table 1shows that plasma TC, TG, LDL-C, VLDL-C and HDL-C of group II were significantly higher as compared to group I. This shows that feeding F-HFD for 30 days induced significant dyslipidemia. Plasma TC, TG, LDL-C, VLDL-C and HDL-C of group III and IV were significantly lower as compared to group II. This shows that administration of isovaleric acid or atorvastatin together with F-HFD prevented dyslipidemia. No significant difference was seen in the plasma TC, TG, LDL-C, VLDL-C and HDL-C of group III and group IV. This means that the anti-dyslipidemic effect of isovaleric acid was comparable to that of atorvastatin.

Table 1 Effects of feeding normal diet, fructose rich-high fat diet (F-HFD), F-HFD+isovaleric acid and F-HFD+ atorvastatin for 30 days on plasma lipids in male Wistar rats

	Group I (Normal diet,n=6)	Group II (F-HFD, n=6)	Group III (F-HFD + isovalericacid, n=6)	Group IV (F-HFD+ atorvastatin, n=6)
Cholesterol (mg/dl)	62.6±9.72	128±18.2[a]	67.3±9.70[x]	68.3±11.6[x]
Triglycerides (mg/dl)	48.1±11.2	110±16.6 [a]	58.6±10[x]	57±4.73[x]
HDL-C (mg/dl)	28.3±8.61	40.5±6.86[b]	29.3±6.25[y]	29.1±6.79[y]
LDL-C (mg/dl)	24.6±19.2	65.5±19.2 [a]	26.2±11.3[x]	27.7±12.3[x]
VLDL-C (mg/dl)	9.6±2.2	22±3.3 [a]	11.7±2.1[x]	11.4±0.94[x]

[a]P<0.001 when compared with group I;[b]P< 0.05 when compared with group I
[x]P<0.001 when compared with group II; [y]P< 0.05 when compared with group II

Table 2 shows the plasma free fatty acids, phospholipids, LCAT and PHLA levels in different groups. No significant difference was found in the phospholipids, LCAT and PHLA levels of group I and group II but plasma free fatty acids were higher in group II. In group III and group IV, plasma free fatty acids, phospholipids and PHLA were significantly lower and LCAT significantly higher as compared to group II. Plasma free fatty acids, phospholipids, LCAT and PHLA levels of group III and group IV were comparable.

Table 2 Effects of feeding normal diet, fructose rich-high fat diet (F-HFD), F-HFD+isovaleric acid and F-HFD+ atorvastatin for 30 days on plasma free fatty acid (FFA), phospholipids (PL), lecithin cholesterol acyl transferase (LCAT) and post heparin lipolytic activity (PHLA) in male Wistar rats

	Group I (Normal diet,n=6)	Group II (F-HFD, n=6)	Group III (F-HFD + isovalericacid, n=6)	Group IV (F-HFD+ atorvastatin, n=6
FFA (mg/dl)	62.5±18.9	90.1±11.6[a]	59.8±12.2[x]	59.1±17.1[y]
PL (mg/dl)	102.3±23.7	128.1±26.3[b]	81±6.48[x]	80.6±3.14[x]
LCAT(nmol/ml/h)	36.3±14.7	44.1±13.4[b]	57.8±10[y]	59.8±10.4[y]
PHLA(nmol/ml/h)	21±3.8	21.6±5.5[b]	12.8±2.63[y]	11.8±2.63[y]

[a]P< 0.05 when compared with group I; [b]P> 0.05 when compared with group I
[x]P< 0.01 when compared with group II; [y]P< 0.05 when compared with group II

Discussion

The aim of the present study was to explore the anti-dyslipidemic activity of a compound isolated from the extract of *V. wallichi*, namely isovaleric acid. Atorvastatin was used as a reference drug for comparison. Dyslipidemia was induced in rats by feeding them fructose rich-high fat diet (F-HFD).

Our results show that: (i) feeding F-HFD resulted in significant dyslipidemia, (ii) both isovaleric acid and atorvastatin were able to prevent dyslipidemia caused by F-HFD and (iii) the anti-dyslipidemic effects of atorvastatin and isovalericacid were comparable. Feeding F-FHD also increased plasma FFA but had no effect on plasma phospholipids, LCAT and PHLA. In the group given isovaleric acid together with F-HFD, plasma FFA, phospholipids and PHLA were significantly lower and LCAT significantly higher as compared to the group given F-HFD alone. Similar results were obtained in the group given atorvastatin together with F-HFD. The effects of atorvastatin and isovaleric acid were comparable.

Thus, isovaleric acid emerges as a potential anti-dylipidemic agent having an efficacy similar to that of atorvastatin, an established anti-dyslipidemic drug. We have not gone into the mechanism of action of isovaleric acid but the change in plasma LCAT activity seems to provide a clue. Studies have revealed a possible association between plasma cholesterol level and LCAT activity.[18,20] Extrahepatic cholesterol is transported to liver by HDL.[18] For this, cholesterol present in other lipoproteins has to be transferred to HDL. This transfer takes place with the help of

LCAT.[19,20] Thus, increased LCAT activity might be one of the reasons for the anti-dyslipidemic effect of isovaleric acid. There might be other reasons too.

One limitation of the present study was its small sample size. Secondly, it is to be seen whether the results obtained in Wistar rats can be replicated in human beings. Safety of isovaleric acid also needs to be established. Yet, the results of the present study show the need for further investigation into the anti-dyslipidemic activity of isovaleric acid in animals and humans.

Conclusion

Isovaleric acid is as effective as atorvastatin in preventing diet-induced dyslipidemia in rats. Further research on its anti-dyslipidemic potential is warranted in animals and humans.

Conflict of interest

The authors declare no conflict of interest.

References

1. Panel ED, Grundy SM. An International Atherosclerosis Society Position Paper: global recommendations for the management of dyslipidemia. Journal of Clinical Lipidology. 2013 Nov 1;7(6):561-
2. ACCORD Study Group. (2010) "Effects of intensive blood-pressure control in type 2 diabetes mellitus." The New England Journal of Medicine 362(17), 1575.
3. Williams RR, Hunt SC, Hopkins PN, Wu LL, Hasstedt SJ, Berry TD, Barlow GK, Stults BM, Schumacher MC, Ludwig EH, Elbein SC. Genetic basis of familial dyslipidemia and hypertension: 15-year results from Utah. American Journal of Hypertension. 1993 Nov 1;6(11_Pt_2):319S-27S.
4. Lalonde L, O'connor A, Joseph L, Grover SA. Health-related quality of life in cardiac patients with dyslipidemia and hypertension. Quality of life research. 2004 May 1;13(4):793-804.
5. Hosseinkhani A, Asadi N, Pasalar M, Zarshenas MM. Traditional Persian Medicine and management of metabolic dysfunction in polycystic ovary syndrome. Journal of Traditional and Complementary Medicine. 2018 Jan 1;8(1):17-23.
6. https://en.wikipedia.org/wiki/3-Methylbutanoic_acid#cite_note-1
7. Eadie MJ. Could valerian have been the first anticonvulsant?.Epilepsia. 2004 Nov;45(11):1338-43.
8. Deeg R, Ziegenhorn J. Kinetic enzymic method for automated determination of total cholesterol in serum. Clin Chem. 1983;29(10):1798-1802.
9. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem. 1973;19 (5):476-482.
10. Burnstein M, Scholnic HR, Morfin R.J. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions.J Lipid Res. 1970 Nov;11(6):583-95.
11. Okada M, Matsui H, Ito Y, Fujiwara A, Inano K. Low-density lipoprotein cholesterol can be chemically measured a new superior method. J Lab Clin. Med. 1998; 132(3):195-201.
12. Hirano T, Ito Y, Saegusa H, Yoshino G. A novel and simple method for quantification of small, dense LDL. J Lipid Res. 2003; 44(11):2193-2201.
13. Knopfholz J,Disserol CC, Pierin AJ, Schirr FL, Streisky L, Takito LL, Massucheto Ledesma P, Faria-Neto JR, Olandoski M, da Cunha CL, Bandeira AM. Validation of the Friedewald formula in patients with metabolic syndrome. Cholesterol. 2014;2014.
14. Monsinger F. Photometric adaptation of dole's microdetermination of free fatty acids. Journal of Lipid Research. 1965;22(8):1206-13.

15. Chan KM, Delfert D, Junger KD. A direct colorimetric assay for Ca2+-stimulated ATPase activity. Analytical Biochemistry. 1986 Sep 1;157(2):375-80.
16. Albers JJ, Chen CH, Adolphson JL. Lecithin: cholesterol acyltransferase (LCAT) mass; its relationship to LCAT activity and cholesterol esterification rate. Journal of Lipid Research. 1981 Nov 1;22(8):1206-13.
17. Wing DR, Robinson DS. Clearing-factor lipase in adipose tissue. A possible role of adenosine 3′, 5′-(cyclic)-monophosphate in the regulation of its activity. Biochemical Journal. 1968 Oct 1;109(5):841-9.
18. Saeedi R, Li M, Frohlich J. A review on lecithin: cholesterol acyltransferase deficiency. Clinical Biochemistry. 2015 May 1;48(7-8):472-5.
19. Dobiášová M. Atherogenic impact of lecithin-cholesterol acyltransferase and its relation to cholesterol esterification rate in HDL (FER (HDL)) and AIP [log (TG/HDL-C)] biomarkers: the butterfly effect. Physiol Res. 2017 May 4;66(2):193-203.
20. Gerl MJ, Vaz WL, Domingues N, Klose C, Surma MA, Sampaio JL, Almeida MS, Rodrigues G, Araújo-Gonçalves P, Ferreira J, Borbinha C. Cholesterol is Inefficiently Converted to Cholesteryl Esters in the Blood of Cardiovascular Disease Patients. Scientific Reports. 2018 Oct 3;8(1):14764.