Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 293–296

4-Hydroxyisoleucine an unusual amino acid as antidyslipidemic and antihyperglycemic agent☆

Tadigoppula Narender,[a,*] Anju Puri,[b] Shweta,[a] Tanvir Khaliq,[a] Rashmi Saxena,[b] Geetika Bhatia[c] and Ramesh Chandra[b]

[a]*Division of Medicinal and Process Chemistry, Central Drug Research Institute, Lucknow, India*
[b]*Division of Biochemistry, Central Drug Research Institute, Lucknow, India*
[c]*Division of Toxicology, Central Drug Research Institute, Lucknow, India*

Received 12 July 2005; revised 23 September 2005; accepted 4 October 2005
Available online 21 October 2005

Abstract—*Trigonella foenum-graecum*, commonly known as fenugreek, is an annual herbaceous plant. From the seeds of *T. foenum-graecum* an unusual amino acid, 4-hydroxyisoleucine **5**, has been isolated, which significantly decreased the plasma triglyceride levels by 33% ($P < 0.002$), total cholesterol (TC) by 22% ($P < 0.02$), and free fatty acids by 14%, accompanied by an increase in HDL–C/TC ratio by 39% in the dyslipidemic hamster model.

According to recent estimates approximately 215 million people all over the world suffer from diabetes mellitus and 80–90% of them are from type II diabetes.[1] This number is expected to increase as medical advances extend life expectancy and more widespread access to a calorie-rich diet promotes the prevalence of obesity. Diabetes mellitus is an independent risk factor for the development of coronary artery diseases, myocardial infarction, hypertension, and dyslipidemia. Clinically, diabetic patients are characterized by marked increase in blood glucose level followed by normal or mild hyperlipidemia. When carbohydrates are in low supply, or their breakdown is incomplete, fats become the preferred source of energy. As a result, fatty acids are mobilized into the general circulation leading to secondary triglyceridemia in which total serum lipids, in particular triglycerides, as well as the levels of cholesterol and phospholipids increase. This rise is proportional to the severity of the diabetes. Uncontrolled diabetes is manifested by a very high rise in triglycerides and fatty acid levels.

An increase in plasma lipids, particularly cholesterol, is a common feature of atherosclerosis, a condition involving arterial damage, which may lead to ischemic heart disease, myocardial infarction, and cerebrovascular incidents. These conditions are responsible for one-third of deaths in industrialized nations.[2] Therefore, a drug, having 2-fold properties, that is, lowering of blood lipids (triglycerides and cholesterol) and glucose together, is in great demand. Several research groups are focusing to develop dual-acting agents some of which (**1** and **2**)[3,4] are in clinical trials.

The discovery of new drugs from traditional medicine is not a new phenomenon. The synthetic biguanides such as Metformin **3** and its analogues[5,6] were synthesized on the basis of natural product leads, that is, galegine **4**, which was isolated from the seeds of *Galega officianalis*.[7] These drugs, which were first marketed in the late 1950s, decrease hepatic glucose production by reducing gluconeogenesis as well as modest improvement in insulin sensitivity. *T. foenum-graecum* (Leguminosae family) is an annual herbaceous plant commonly known as fenugreek and is widely distributed across Asia, Africa, and Europe. Fenugreek seeds are of value to the steroid industry and have special culinary uses.[8] Several groups[9–16] have reported antihyperglycemic activity in its alcoholic and aqueous extracts. Fowden[17] was the first to isolate and identify the unusual amino acid, 4-hydroxyisoleucine **5**. It was estimated that **5**

Keywords: 4-Hydroxyisoleucine; Antidyslipidemic activity; Hamster model.
☆ CDRI Commun. No. 6689.
* Corresponding author. Tel.: +91 0522 2612411x17; fax: +91 0522 2623405/2623938; e-mail: tnarender@rediffmail.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.10.003

accounted for 80% of the total amino acid within the seeds. Christophe et al.[18] discovered that the major isomer, that is, 2*S*,3*R*,4*S* of 4-hydroxyisoleucine induces insulin secretion through a direct effect on pancreatic β cells in rats and humans. Furthermore, in a new model of type II diabetes rats, the above compound was active and partly corrected hyperglycemia and glucose tolerance.[19] Sharma et al.[20] reported the effect of fenugreek seeds on lowering of serum lipids for the first time. A clinical study was also carried out on the effect of fenugreek on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease.[21] Recently, the soluble dietary fiber fraction of *T. foenum graecum* has been reported for its effect on glycemic, insulinemic, lipidemic, and platelet aggregation in a type II diabetes model.[22] However, the lipid-lowering principle has not been identified in their studies.[20–22] Our-activity guided fractionation and isolation work led to discover the antidyslipidemic activity for the first time in the compound **5**.

In the present study, we carried out experiments to investigate the antidyslipidemic activity of **5** in the high fat diet (HFD) fed dyslipidemic hamster model, which has been reported as an ideal in vivo model for evaluating antidyslipidemic drugs.[23,24] Feeding with HFD in hamster induced dyslipidemia as the plasma levels of triglycerides (TG),[25] total cholesterol (TC),[26] high density lipoprotein–cholesterol (HDL–Chol),[27] glycerol (Gly)[28] and free fatty acids (FFA)[29] were found to be significantly elevated by 5-, 2.8-, 1.7-, 1.4- and 5.3-fold, respectively, as compared to normal chow fed control animals (Table 1).

A purified compound **5**, isolated from the fenugreek seeds, was administered orally at the dose of 50 mg/kg b wt for seven consecutive days. No significant differences were observed in the food intake and weight gain between the groups. Results obtained in dyslipidemic hamsters showed a significant decrease in lipid profile. The higher TG levels and lower HDL–C increase the risk of coronary heart disease (CHD).[30] The HDL–C mediate the reverse transport of cholesterol from peripheral tissues to the liver for disposal by excretion into bile. This process will disallow the slow accumulation of lipids in artery walls. The compound **5** has exhibited both the above-mentioned properties. It significantly lowered the plasma triglycerides from 9.73 to 6.56 mM by 33%, total cholesterol (TC) by 22% and increased the HDL–C from 2.30 to 2.52 mM by 8.7% similar to finofibrate's **6**[31,32] action (Fig. 1). The compound **5** also lowered the free fatty acids (FFA) by 14% and glycerol by 4.9% compared to HFD fed hamsters. The ratio between HDL–C and total cholesterol (TC) was increased by 39% after the treatment with the compound **5**, which is considered a beneficiary effect in the treatment of dyslipidemia condition.

In conclusion, we have isolated an unusual amino acid **5**, which has antidyslipidemic activity in the hamster model from *T. foenum-graecum* seeds. Earlier, the compound **5** was reported for its antidiabetic activity in type II diabetes model, hence the compound **5** may be considered as a novel lead, which could be a potential antidiabetic and antidyslipidemic agent. Synthesis of appropriate analogues may pave the way to develop a potent dual-acting drug.

Isolation of 4-hydroxyisoleucine ***5***. The seeds (5 kg) of *T. foenum-graecum* were collected from the local market of Lucknow and extracted with 4 l of ethyl alcohol four times in a percolator. The resultant alcoholic extract was (16 l) combined and concentrated under reduced pressure to give 100 g of alcohol extract. This was fractionated with chloroform and *n*-butanol successively. The resultant aqueous fraction (10 g) was subjected to conventional silica gel column chromatography using ethyl acetate and methanol (90:10) solvent system to give an unusual amino acid (750 mg). It was characterized as 4-hydroxyisoleucine by using ^{1}H NMR, ^{13}C NMR, IR

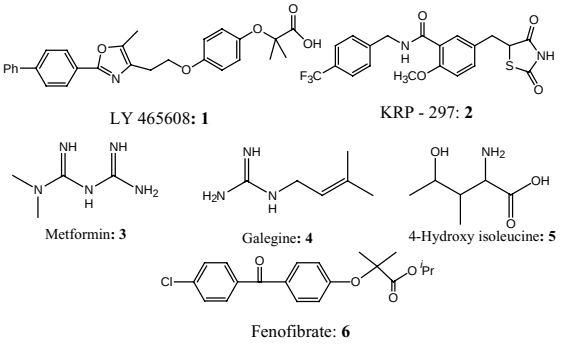

Figure 1. PPARα/γ dual agonist in clinical trials, **1** and **2**; Synthetic antidiabetic agent, **3**; Naturally occuring antidiabetic agents, **4** and **5** and triglyceride lowering drug, **6**.

Table 1. Effect of compound **5** on biochemical parameters in dyslipidemic hamsters at 50 mg/kg body weight (values are means ± SD of eight hamsters in each group)

	Control	HFD	HFD + 4-hydroxyisoleucine
TG (mM)	1.92 ± 0.61	9.73 ± 2.93	6.56 ± 0.81 (−33)**
TC (mM)	3.44 ± 0.77	9.87 ± 1.37	7.75 ± 2.31 (−22)*
HDL–C (mM)	1.35 ± 0.16	2.30 ± 0.46	2.52 ± 0.42 (+9)
Gly (mM)	0.70 ± 0.19	1.01 ± 0.25	0.96 ± 0.32 (−4)
FFA (μM)	151 ± 17.5	802 ± 264	689 ± 147 (−14)
HDL–C/TC	0.39	0.23 (−41)	0.32 (+39)
Food intake (g)	8.50 ± 1.25	7.13 ± 1.5	6.97 ± 1.39
Body weight (g)	125.7 ± 5.50	131.0 ± 5.15	126.4 ± 7.98

P values: *<0.02; **<0.002.

Values in parentheseis indicate the increase or decrease as compared to control HFD fed animals.

and mass spectral data and comparing with literature data. The HPLC analysis of the isolated compound indicated the presence of minor isomer, that is, 2*R*,3*R*,4*S* (6%) along with major isomer 2*S*,3*R*,4*S* (94%).

IR: 3139, 1632 cm^{-1}, ^1H NMR in D_2O: δ 0.93 (3H, d), 1.22 (3H, d), 1.89 (1H, m), 3.85 (2H, m); ^{13}C NMR in D_2O: δ 12.5 (CH_3), 21.1 (CH_3), 41.6 (CH), 57.3 (CH), 70.2 (CH), 174.1 (CO); FAB Mass 148 (M+1).

Experimental animals. Golden Syrian hamsters, male, 12 weeks old, 110–120 g body weight were used. A group of eight animals were kept in controlled conditions, temperature 25–26 °C, relative humidity 60–80%, and 12/12 h light/dark cycles (light from 8.00 a.m. to 8.00 p.m.). The animals with identification marks were acclimatized for 7 days before experiment.

High fat diet (HFD). The HFD was purchased from Research Diet Inc., New Breenswick, USA (Product code No. 99/12211). This diet contained normal pellet diet mixed with groundnut oil, cholesterol (Sigma), deoxycholate (Sigma), and fructose (Sigma) in a ratio of 610:300 ml:5 g:5 g:100 g, respectively, to contain 1 kg HFD. This homogeneous soft cake was molded in the shape of pellets of about 3 g each. Animals of all the groups, except control, were fed with HFD (10 g/animal) daily from day one to day ten. Control group received the normal hamster chow. Animals had free access to the diet and water. The daily diet consumed by animals was calculated by subtracting the leftover diet on next day from the added diet previous day. The body weight of each animal was recorded daily. Drug was administered orally (suspension in gum of acacia); daily once for seven consecutive days (day 4 to day 10) to treated HFD groups. HFD fed and normal chow fed control groups were given the same amount of vehicle.

Collection of blood samples and biochemical analysis from plasma. The blood was collected on day 10, 2 h after the last drug administration. Hamster (nonfasted) were anesthetized by pentothol sodium (50 mg/ml in normal saline), injection (1 ml/kg b wt) ip Blood was withdrawn from retroorbital plexus using 10 μl (20 mm (L) × 0.8 mm (dia) glass capillary and collected in an EDTA coated-tube (3 mg/ml) for the estimation of total cholesterol (TC),[26] triglyceride (TG)[25] and HDL–cholesterol (HDL–C).[27] Blood was collected in separate EDTA-coated tubes, containing 5.4 mg NaF (45 mg/ml) in 120 μl volume, for free fatty acid (FFA) (Wako Pure Chemicals) and glycerol (Gly)[28] estimation. Blood was kept in wet ice for 30 min, centrifuged at 4 °C, and plasma was separated for estimation. The analysis of plasma biochemical parameters was performed on auto-analyzer of Beckman 'Coulter model Synchron CX-5 clinical system,' by standard enzymatic methods. The assay kits were purchased from Beckman Coulter, International, USA, except for FFA, which was purchased from Wako Pure Chemicals Industries, Limited, Osaka, Japan.

Statistical analysis: Data were analyzed by Student's *t* test.

The percentage of lowering of lipids calculated as below:

%lowering = HFD animal lipid values − drug + HFD animal lipid values/HFD animal lipid values × 100.

Acknowledgments

Authors are grateful to Dr. C. M. Gupta, Director, CDRI, Lucknow, India, for constant encouragement for the program on antihyperglycemic and antidyslipidemic drugs. We also thank the RSIC for spectral data, J. P. Chaturvedi for technical support, and botany division of CDRI for material supply. Tanvir and Shweta are thankful to ICAR, and DOD, New Delhi, respectively, for financial support.

References and notes

1. Bennett, P. H. *Diabetes Metab. Rev.* **1997**, *13*, 583.
2. Eghdamian, E.; Ghose, K. *Drugs Today* **1998**, *34*, 943.
3. Etgen, G. J.; Oldham, B. A.; Johnson, W. T., et al. *Diabetes* **2002**, *51*, 1083.
4. Nomura, M.; Kinoshita, S.; Satoh, H.; Maeda, T.; Murakami, K.; Tsunoda, M.; Miyachi, H.; Awano, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 533.
5. Werner, E. A.; Bell, J. *J. Chem. Soc.* **1922**, *221*, 1790.
6. Shapiro, S. L.; Parrino, V. A.; Freedman, L. *J. Am. Chem. Soc.* **1959**, *81*, 3728.
7. Bailey, C. T. *Diabetes Care* **1989**, *12*, 553.
8. Fazli, F. R. Y.; Hardman, R. *Phytochemistry* **1971**, *10*, 2497.
9. Ajabnoor, M. A.; Tilmisay, A. K. *J. Ethanopharmacol.* **1988**, *22*, 45.
10. Amin, R.; Abdul Ghani, A. S.; Suleiman, M. S. *Planta Med.* **1988**, *54*, 286.
11. Madar, Z.; Abel, R.; Samish, S.; Arad, J. *Eur. J. Clin. Nutr.* **1988**, *42*, 51.
12. Madar, Z.; Arad, J. *Nutr. Res.* **1985**, *9*, 691.
13. Sauvaire, Y.; Petit, B.; Broca, C.; Mantghetty, M.; Baissae, Y.; Fernandez-Alvarez, J.; Gross, R.; Roye, M.; Leconte, A.; Gomis, R.; Ribes, G. *Diabetes* **1988**, *47*, 206.
14. Shani, J.; Goldschmied, A.; Joseph, B.; Aharonson, Z.; Sulman, F. G. *Arch. Int. Pharmacodyn. Ther.* **1974**, *210*, 27.
15. Madar, Z.; Stark, A. H. *Br. J. Nutr.* **2002**, *88*, 287.
16. Broca, C.; Breil, V.; Cruciani, G. C., et al. *Am. J. Physiol. Endocrinol. Metab.* **2004**, *287*, 463.
17. Fowden, L.; Pratt, H. M.; Smith, A. *Phytochemistry* **1973**, *12*, 1707.
18. Christophe, B.; Manteghetti, M.; Gross, R.; Baissac, Y.; Jacob, M.; Petit, P.; Sauvaire, Y.; Ribes, G. *Eur. J. Pharmacol.* **2000**, *390*, 339.
19. Broca, C.; Gross, R.; Petit, P.; Sauvaire, Y.; Manteghetti, M.; Tournier, M.; Masiello, P.; Gomis, R.; Ribes, G. *Am. J. Physiol.* **1999**, *277*, E617.
20. Sharma, R. D.; Raghuram, T. C.; Rao, N. S. *Eur. J. Clin. Nutr.* **1990**, *44*, 306.
21. Bordia, A.; Verma, S. K.; Srivastava, K. C. *Prostaglandins, Leukot. Essent. Fatty Acids* **1997**, *56*, 379.
22. Hannan, J. M. A.; Rokeya, B.; Faruque, O.; Nahar, N.; Mosihuzzaman, M.; Azad Khan, A. K.; Ali, L. *J. Ethanopharmacol.* **2003**, *88*, 73.
23. Rizvi, R.; Puri, A.; Bhatia, G.; Khanna, A. K.; Wulf, E. M.; Rastogi, A. K.; Chandra, R. *Biochem. Biophys. Res. Commun.* **2003**, *305*, 215.

24. Bhatia, G.; Rizvi, F.; Saxena, R.; Puri, A.; Khanna, A. K.; Chander, R.; Wulf, E. M.; Chandra, R.; Rastogi, A. K. *Indian J. Exp. Biol.* **2003**, *41*, 1456.
25. Buccolo, G.; David, H. *Clin. Chem.* **1973**, *19*, 476.
26. Deeg, R.; Ziegenhorn, J. *Clin. Chem.* **1983**, *29*, 1798.
27. Gordon, D. J.; Probstfield, J. L.; Garrison, R. J.; Neatom, J. D.; Jacob, D. R.; Bangdiwala, S., Jr.; Tyroler, H. A. *Circulation* **1989**, *79*, 8.
28. Gowan, M. W.; Artiss, J. D.; Strandbergh, D. R.; Zak, B. *Clin. Chem.* **1983**, *29*, 538.
29. Wako pure Chemical Limited. NAFAC, Quantitative determination of free fatty acid in serum by ACS–ACO Method Company code No. 994-75409E.
30. Elokdah, H.; Sulkowski, T. S.; Gharbia, M. A.; Butera, J. A.; Chai, S. Y.; McFarlane, G. R.; McKean, M. C.; Babiak, J. L.; Adelman, S. J.; Quinet, E. M. *J. Med. Chem.* **2004**, *47*, 681.
31. Issemann, I.; Green, S. *Nature* **1990**, *347*, 645.
32. Koyama, H.; Miller, D. J.; Boueres, J. K.; Desai, R. C., et al. *J. Med. Chem.* **2004**, *47*, 3255.