Nutritional Sciences 7(4), November, 2004, pp.214-217

Effects of Dietary Intervention and Simvastatin on Plasma Nitric Oxide in Patients with Hyperlipidemia

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Dietary intervention and simvastatin is beneficial in the prevention cardiovascular diseases by lowering plasma lipid levels. Endothelial dysfunction is associated with coronary artery disease and its risk factors and is reversed by dietary intervention. It has been suggested that hyperlipidemia contributes to the development of atherosclerosis by increasing inducible nitric oxide synthase (iNOS) expression via intimal thickening. Statins treatment has been found to decrease iNOS expression and atherogenensis in animal models. We hypothesized that dietary intervention and simvastatin therapy could decrease plasma nitric oxide in hypercholesterolemic patients, which would suggest the opportunity for modulation of iNOS expression through the use of statins in a clinical situation. We measured the plasma levels of nitrite and nitrate (NOx) in 19 hyperlipidemia patients. The subjects were under dietary intervention following simvastatin therapy for 12 weeks. As a result, the plasma level of NOx, stable metabolites of nitric oxide (NO), saw a two-fold elevation in hyperlipidemic patients as compared to normal levels. Although 12 weeks of dietary intervention did not lower NOx levels, subsequent 12-week simvastatin (10 mg/day) treatment, along with dietary intervention, lowered NOx levels significantly. This NOx reduction, induced by simvastatin therapy, positively correlated with lowered coronary risk factors ($r=0.40$, $p=0.02$). It indicated that simvastatin therapy decreases plasma NOx levels by, perhaps, decreasing iNOS expression or activity leading to the attenuation of the development of neointima.

Key words: Hyperlipidemia, Nitric oxide, Simvastatin, iNOS

INTRODUCTION

Hyperlipidemia is known to be the central pathogenic factor that causes atherosclerosis in various arteries, thus leading to coronary disease. For patients with hyperlipidemia, dietary intervention and inhibitors of 3-hydroxy-3-methylglutaryl coenzme A (HMG-CoA) reductase such as simvastatin (statins) are widely used to lower plasma cholesterol levels and improve the survival of coronary disease patients.

A consequence of endothelial damage is a lower availability of nitric oxide (NO), the most potent endogenous vasodilator. NO inhibits platelet aggregation, smooth muscle cell proliferation and the adhesion of monocytes to endothelial cells.[1)]

Dietary factors may induce significant changes in vascular reactivity. There is evidence of the significant role of diet on endothelial function and its impact on the pathogenesis of atherosclerosis.[2)] Endothelial dysfunction is associated with coronary artery disease and its risk factors and is reversed by antioxidants and marine n-3 fatty acids.[3)] Eicosapentaenoic acid can make an additional improvement in the mortality and morbidity of coronary artery disease beyond that of HMG-CoA reductase inhibitor treatment.[4)]

In addition to the cholesterol-lowering effect, experimental studies have shown that statins influence NOS activity and expression.[5)] In rabbits fed cholesterol-enriched diet, the development of atherosclerotic lesions in several arteries was accompanied by decreased endothelial nitric oxide synthase (eNOS) activity, decreased endothelium dependent relaxation and positive immuno-histochemical staining of inducible nitric oxide synthase (iNOS).[6)] Moreover, statin therapy is known to facilitate a recovery in eNOS activity and expression and decrease iNOS expression and its correlated intimal thickening in animal studies.[7-9)]

However, it is not known whether dietary intervention and statins inhibit NO production or decrease iNOS expression in humans. And based on existing experiments, it is difficult to predict the change in plasma NO concentration of hypercholesterolemia patients after dietary intervention

Accepted : November 23, 2004
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and statin therapy. Dietary intervention and statin therapy leads to the activation of eNOS activity in the endothelium, while suppressing iNOS expression in the leukocytes infiltrating the atherosclerotic lesions.

The objective of the study was to clarify the effect of dietary intervention and simvastatin therapy on plasma NO concentration in hyperlipidemia, the lipid profile and coronary risk factors.

METHOD

1. Subjects and Experimental Design

The subjects were 19 hyperlipidemic patients (4 males and 15 females) whose plasma triglyceride and total cholesterol levels were >200 mg/dL, as measured at the Medical Examination Center of K university. Mean age of the 19 hyperlipidemic subjects was 58.2 years old. Weight value for the males was 66.7±5.4 kg and 59.2±4.2 kg for the females. The figure for ideal body weight percentage was 102.5% for the males and 117.3% for the females. All of the subjects voluntarily entered the dietary intervention and simvastatin therapy. The therapy was composed of a 12-week dietary intervention and subsequent 12 weeks of simvastatin (10 mg/day) administration along with the dietary intervention. Simvastatin therapy and dietary intervention were conducted according to the Korean guidelines for the treatment of hyperlipidemic patients. Thirty-six normal subjects were selected from among medical school students at I university, whose plasma triglyceride and total cholesterol levels were < 200 mg/dL.

2. Dietary Intervention

During the 12-week dietary intervention, subjects visited once every 2 weeks. The subjects were instructed to keep to hypolipidemic and hypocholesterolemic diets. The dietary intervention involved the substitution of white meat and fish for fatty and red-meat products; increasing the consumption of vegetables, legumes and fruit; reducing egg consumption; and avoiding sweets and pastries. Nutrition intake was determined, before and after the 12-week dietary intervention, using the 24 hr recall method. The records of foods eaten were assessed through personal interviews at two-week intervals. Data were analyzed using the nutritional analysis program (CAN pro, The Korean Nutrition Society, Korea).

3. Biochemical Assays

Blood samples for biochemical assays were obtained after overnight fasting before, during (12th week) and after (24th week) simvastatin therapy. Plasma levels of lipids (triglycerides, cholesterol, HDL-cholesterol) were quantified using the enzymatic method on Reflotron system automated analyzers (Boehringer Manheim). Plasma levels of LDL cholesterol were calculated using Fredewald's formula. Apolipoprotein A-1 and apolipoprotein B were determined using immunonephelometry on a Kallestad QM 300 system. Based on the results collected, the atherogenic index (AI) and coronary risk factor (CRF) were calculated (Table 1).[10,11]

Table 1. The effects of dietary intervention on nutrient intakes in patients with hyperlipidemia

	Before dietary intervention	After dietary intervention+
Calories (kcal)	1878.1±83.8	1523.2±29.5**
Carbohydrate (%)	64.5±1.5	65.2±0.7
Protein (%)	16.1±0.5	16.3±0.3
Fat (%)	18.6±0.9	18.0±0.5
Fat (g)	40.1±3.0	30.7±1.1*
Cholesterol (mg)	182.7±32.5	139.2±9.5

Values are Mean±SEM
+Values are mean intakes of 12 weeks during dietary intervention
*p<0.05; **p<0.01 (vs. before dietary intervention, paired *t*-rest)

4. Determination of Plasma NO Production

The concentration of stable NO metabolites (nitrite/nitrate; NOx) present in the plasma at the time of sampling was determined.[12] After converting the plasma nitrate (NO^{3-}) to nitrite (NO^{2-}) using nitrate reductase (10 U/mL in 100 M Tris buffer, pH 7.6), the total concentration of nitrite was determined spectrophotometrically at 560 nm using a method based on the Griess reaction.

5. Statistical Analysis

Results are expressed as mean±SE. Two-way ANOVA with repeated measures was used to compare simvastatin and placebo treatments. When a significant difference was revealed using this analysis, comparisons at each drug infusion level were made using two-tailed t tests. A value of $p<0.05$ was considered significant.

RESULTS

1. The Effects of Dietary Intervention on Clinical Characteristics and Nutrient Intakes

After dietary intervention, weight values for the males were 64.0±5.8 kg and those for the females were 57.2±3.0 kg. The 12-week dietary intervention did not significantly alter the weight values.

As the results in Table 1 show, dietary intervention significantly lowered calorie intakes compared with the basal diet. The patients moved from a basal diet containing 19% fat (182.7 mg cholesterol/day), 16% protein and 84% carbohydrate to a diet containing 18% fat (139.2 mg cholesterol/day), 16% protein, and 65% carbohydrate for 12 weeks.

Table 2. The effects of dietary intervention and simvastatin therapy on plasma lipid profiles, nitric oxide levels and coronary risk factor in patients with hyperlipidemia

Lipid profiles	Untreated Patients	After 12 weeks of Dietary intervention	p value	After 12 weeks of Dietary intervention+Simvastatin	p value
TG	214.40±147.99	201.04±115.72	0.65	157.16±89.99	0.11
TC	248.74±35.57	252.37±48.29	0.73	222.74±50.96	0.03*
LDL	162.28±42.78	163.08±49.33	0.94	133.56±44.07	0.02*
HDL	43.58±14.68	49.08±15.21	0.06	57.55±20.39	0.01**
AI	5.30±2.03	4.57±1.75	0.05*	3.26±1.67	0.0005**
Apo A-I	153.42±25.57	154.32±28.55	0.39	165.31±29.47	0.03*
Apo B	113.75±19.25	109.87±15.82	0.39	97.24±35.73	0.03*
Apo B/A-I	0.76±1.16	0.73±0.14	0.39	0.60±0.22	0.03*
Coronary Risk Factor	15.63±12.06	12.85±14.13	0.12	9.54±10.39	0.02*
NOx	83.15±49.47	77.38±34.74	0.69	56.33±31.61	0.05*

AI (Atherogenic Index): TC+HDL/HDL, NOx: NO_2-+NO_3-, *p<0.05; **p<0.01 (vs. untreated patients, paired *t*-rest)

2. The Effects of Dietary Intervention on Plasma Lipid Profiles, Nitric Oxide Levels and Coronary Risk Factor

As the results in Table 2 show, 12 weeks on a low-cholesterol diet did not significantly alter the plasma levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). Similarly, the diet therapy did not significantly change the plasma levels of apolipoprotein A-1 (apo A-1) and apolipoprotein B (apo B). There was no change in the coronary risk factor (CRF), which was calculated using the PROCAM Risk Calculator pro- gram,[3] but the atherogenic index (AI) appeared to have decreased significantly (p=0.05) as a result of the diet therapy. Similarly, the elevated mean NOx value in the hyperlipidemic patients (83.15±49.47 μM), which in the normal volunteers was 37.9±30.95 μM, did not decrease (Fig. 1).

3. The Effects of Additional Simvastatin Therapy On Plasma Lipid Profiles, Nitric Oxide Levels and Coronary Risk Factors

The results also showed that 12 weeks of simvastatin therapy decreased plasma levels of TC, LDL-C and apo B, all significantly, without affecting the TG level. Conversely, the plasma levels of HDL-C and apo A-1 increased significantly. These results contributed to the reduction in AI and CRF. Most significantly, simvastatin therapy lowered plasma NOx levels to 57.19±31.42 μM. The reduction in the level of plasma NOx achieved through simvastatin therapy positively correlated with the lowered CRF (r=0.40, p=0.02).

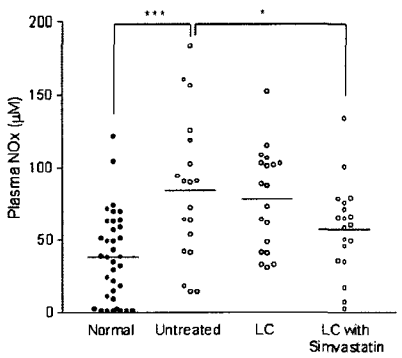

Fig. 1 The effects of low-cholesterol diet (LC) and simvastatin therapy on plasma NOx concentrations in hyperlipidemic patients. Horizontal bars represent median values.
***p<0.0001, *p<0.05.

DISCUSSION

In a hyperlipidemic condition, endothelial dysfunction caused by reduced eNOS activity, decreased vascular relaxation and increased leukocyte-endothelial cell interaction is commonly observed.[13] This promotes atherosclerosis at high sheer-stress sites where fatty streaks, macrophage infiltration and smooth muscle cell proliferation are observed. Although the constitutive eNOS is known to produce only small amounts of NO intermittently, with reduced eNOS activity in the vast number of systemic endothelial cells, there are expectations of a lowering of the NOx level. However, the plasma NOx was elevated in hyperlipidemic patients compared to normal subjects who were not matched in age (Fig. 1). Though further research is necessary, this result may be explained by the expression of iNOS, which is known to produce large amounts of NO continuously, in human atherosclerotic lesions. While it was not possible to assess the extent of atherosclerotic lesions present in our patients, this increased plasma NOx may have originated from the infiltrated macrophages or vascular smooth muscle cells expressing iNOS at many unkown sites.[14-16] In the face of reduced eNOS activity, ample NO is still required to maintain vasodilation with the inhibition of platelet aggregation and for the suppression of further leukocyte adhesion to the endothelium, further oxidation of LDL

by scavenging oxygen radicals and further proliferation of vascular smooth muscle cells.[17] Thus, the elevated plasma NOx observed in hyperlipidemic patients may represent a defense mechanism of nitric oxide provided by the iNOS expressing cells or the subsequent pathological phenomenon of hyperlipidemia leading to intimal thickening because of overproduced nitric oxide.

Connor *et al.*[18] found that the n-3 fatty acids in fish and fish oil have great potential in the prevention and treatment of patients with coronary artery disease. The n-3 fatty acids promote the synthesis of the beneficial nitric oxide in the endothelium. In this study, we demonstrated that dietary intervention had no effect on plasma nitric oxide levels. The ineffectiveness of the dietary intervention may have been due to the limited changes in energy intake and cholesterol intake without an n-3 ratio change.

Statin therapy, widely administered to lower plasma cholesterol levels in hyperlipidemic coronary heart disease patients, has recently been shown to reverse endothelial dysfunction by increasing NO production from endothelium.[8] Furthermore, in the atherosclerotic rabbit model in which the process was induced through the feeding of a cholesterol-enriched diet, simvastatin was recently reported to inhibit the expression of iNOS in atherosclerotic lesions and to arrest the progression of atherosclerosis.[7] This may explain the reduction in plasma NOx levels observed after the simvastatin therapy (Fig. 1). Therefore, dietary intervention had no effect on plasma nitric oxide levels. But simvastatin therapy appeared to selectively decrease iNOS expression, which produced large amounts of NO and determined overall plasma NOx concentration while increasing eNOS activity and expression, which may not contribute to plasma NOx levels in hyperlipidemic patients.

Literature Cited

1) Huang PL. Endothelial nitric oxide synthase and endothelial dysfunction. *Curr Hypertens Rep* 5(6):473-480, 2003
2) Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giguliano G, D'Armiento M, D'Andrea F, Giugliano D. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 292(12):1490-1492, 2004
3) Ros E, Nunez I, Perez-Heras A, Serra M, Gilabert R, Casals E, Deulofeu R. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. *Circulation* 109(13):1609-14, 2004
4) Yokoyama M, Origasa H, JELIS. Investigators Effects of eicosapentaenoic acid on cardiovascular events in Japanese patients with hypercholesterolemia: rationale, design, and baseline characteristics of the Japan EPA Lipid Intervention Study (JELIS). *Am Heart J* 146(4):613-20, 2003
5) Davignon J, Laaksonen R. Low-density lipoprotein-independent effects of statins. *Curr Opin Lipidol* 10:543-559, 1999
6) Behr D, Rupin A, Fabiani JN, Verbeuren TJ. Distribution and prevalence of inducible nitric oxide synthase in atherosclerotic vessels of long-term cholesterol-fed rabbits. *Atherosclerosis* 142:335-344, 1999
7) Jose A, Joan FG, Maria B, and Lina B. Nitric oxide synthase II (NOS II) gene expression correlates with atherosclerotic intimal thickening. Preventive effects of HMG-CoA reductase inhibitor. *Atherosclerosis* 145:325-331, 1999
8) Ulrich L, Vito LF, Jorge P, James KL. Upregulation of Endothelial Nitric Oxide Synthase by HMG-CoA reductase inhibitors. *Circulation* 97:1129-1135, 1998
9) Mital S, Zhang X, Zhao G, Bernstein RD, Smith CJ, Fuulton DL, Sessa WC, Liao JK, Hintze TH. Simvastatin upregulates coronary vascular endothelial nitric oxide production in conscious dogs. *Am J Physiol Heart Circ Physiol.* 279(6): H2649-57, 2000
10) Cullen P, Von Eckardstein A, Assmann G. Diagnosis and management of new cardiovascular risk factors. *Eur Heart J* 19 Suppl. O:13-19, 1998
11) Assmann G, Schulte H, Von Eckardstein A. Hypertriglycemia and elevated lipoprotein(a) are risk factors for major coronary events in middle-aged men. *Am J Cardiol* 77:1179-84, 1996
12) Green LC. Analysis of nitrate, nitrite, and [N15] nitrate in biological fluids. *Anal Biochem* 125:131-138, 1982
13) Pruefer D, Scalia R, Lefer AM. Simvastatin inhibits leukocyte-endothelial cell interations and protects against inflammatory processes in normocholesterolemic rats. *Arterioscler Thromb Vasc Biol* 19(12):2894-2900, 1999
14) Hibbs JJ, Tainor, Vavrin Z, Rachlin EM. Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun* 157:87-94, 1998
15) Radomski MW, Palmer RMJ, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci* 87:10043-7, 1990
16) Knowles RG, Merrett M, Salter M, Moncada S. Differential induction of brain, lung and liver nitric oxide synthase by endotoxin the rat. *Biochem J* 270:833-6, 1990
17) Andreas HW, Thomas K, Uwe R, Ingo J, Markus H. Improvement of nitric oxide dependent vasodilation through attenuation of endothelial superoxide anion formation. *Arterioscler Thromb Vasc Biol* 20:61-69, 2000
18) Connor SL, Connor WE. Are fish oils beneficial in the prevention and treatment of coronary artery disease? *Am J Clin Nutr* 66(4 Suppl):1020S-1031S, 1997