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高密度脂蛋白過低病人治療前後單核細胞與內皮細胞接合之研究

Study on mononuclear cell adhesion to endothelial cells in patients with low serum high-density lipoprotein levels before and after interventionsAbstract

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目錄

Abstract

Key words: high density lipoprotein cholesterol, felofibrate, atherosclerosis, cell adhesion, human umbilical vein endothelial cell

In order to examine the possible beneficial effects of elevating the initially low high density lipoprotein cholesterol (HDL-C), patients mainly with low HDL-C were included in this study. Patients with HDL-C <40 mg/dl with normal or mild elevation of cholesterol (CH, <250 mg/dl) and/or normal or mild to moderate elevation of triglyceride (TG, <400 mg/dl) were studied with lipid profile measurements and cell adhesion measurements. Then they were treated with life style modification, and drug therapy if necessary, to elevate the HDL-C level.

Totally 59 patients (42 males), with mean ages of 65.4±9.8 years were studied. Most of the patients (45 cases, 76.3%) received fenofibrate treatment. The lipid profile follow-up showed no changes in CH and LDL-C levels after intervention while TG was significantly reduced (averaged -28%) and HDL-C markedly increased (averaged +18.7%). Cell adhesion was significantly reduced after treatment ($p<0.0001$).

It is concluded that by intensive interventions on patients with low HDL-C to raise HDL-C levels with concomitant reduction in TG levels, the mononuclear cell adhesion to the cultured HUVEC was demonstrated to decrease significantly. The clinical significance of these findings needs further studies.

Cardiovascular disease is the most important cause of of morbidity and mortality in the modern life. The importance of cardiovascular diseases is rapidly increasing in our country in past decades, as demonstrated by the life statistics. Atherosclerosis would affect different arteries, resulting in coronary artery disease, cerebrovascular disease, aortic disease and various peripheral vascular disorders.

The earliest event in the process of atherosclerosis is adhesion of circulating mononuclear cells, especially monocyte, to the endothelial lining of the vessel (1-3). The adhered mononuclear cells then transmigrate across the endothelium to the subintimal layer where they replicate and, by engulfing lipids, transform into foam cells (1-3). These changes resulted in fatty streak which is the earliest pathological manifestation of atherosclerosis. Therefore, cell adhesion is the first step in the process of atherosclerosis. The studies about adhesion of mononuclear cells to endothelium is now one of the most active fields of research in the scope of atherosclerosis.

Endothelial dysfunction plays an important role in the pathogenesis of atherosclerosis (1-4). Many risk factors for the development of atherosclerosis have been identified in the past decades, among them are hypertension, dyslipidemia, smoking, diabetes mellitus, and obesity. (5). It has been reported that hypertension (6-8), hypercholesterolemia (9-13), hypertriglyceridemia (9,11,13), and diabetes mellitus (15-21) all enhanced mononuclear cell adhesion to endothelial cells in culture and/or vascular endothelium.

It has been reported that low serum high density lipoprotein cholesterol (HDL-C) is especially important as a coronary risk factor for Chinese in Taiwan (22,23). The importance of HDL-C has been especially emphasized in the recently released ATP III (24). In the new recommendation, the lower limit of HDL-C level has been raised to 40 mg/dl, instead of 35 mg/dl, for males. Yet, as to our knowledge, it is worth to note that there is no study concerning the effects of serum HDL-C on the adhesion of monocytes or mononuclear cells to endothelial cells or vascular endothelium in the literature.

We therefore, proposed to study the blood mononuclear cell adhesion to the cultured human umbilical vein endothelial cells (HUVEC) in patients with low HDL-C. We further investigated the effects of HDL-C elevation after intervention on the cell adhesion in these patients.

Subjects and Methods

1. Culture of HUVEC

The HUVEC will be obtained and cultured following the method of Jeffe et al (25). The cells will be cultured with M199 supplemented with 20% fetal bovine serum, 20 mM HEPES, 100 μg/ml endothelial cell growth substance (Collaborative Research, Bedford, MA), 5 U/ml heparin, 100 IU/ml penicillin and 0.1 mg/ml streptomycin (26). Subculture is undertaken when confluence of the cell occurs, by applying 0.25% trypsin-EDTA (Gibco, Gaithersburg, MD) for 3 minutes. Cells of 3rd to 6th passage will be used in experiments.

2. Study subjects

Subjects with low HDL-C (<40 mg/dl) were included in this study. Their total cholesterol should be <250 mg/dl and triglyceride <400 mg/dl. Patients with hypertension and diabetes mellitus were excluded. Smoking and/or obesity were not excluding factors, but were factors for intervention to increase HDL-C.

After initial studies on mononuclear cell adhesion, the subjects were evaluated for possible conditions for intervention to increase the HDL-C. These conditions might include obesity, smoking, lack of exercise, etc. In patients whose HDL-C did not elevate to ≥40 mg/dl, drugs, especially fibrates, were prescribed. After 3 months or longer of interventions, the serum HDL-C levels were rechecked. In case HDL-C elevated to ≥40 mg/dl or increase > 3 mg/dl (about 10%), the mononuclear cell adhesion studies were repeated to evaluate the effects of HDL-C elevation.

3. Isolation of plasma and mononuclear cells

Fasting venous bloods from patients subjects were collected in tubes containing EDTA. The blood samples were then centrifuged at 2000 rpm for 15' and the plasmas were obtained and stored at 4 degree C for later analysis.

To isolate mononuclear cells from the patients and the controls, the method of Menon et al. was used (27). Anticoagulated human blood was layered over an equal volume of Histopaque 1077 (Sigma), and then centrifuged at 400 x g for 30' at room temperature. Mononuclear cells are collected from the interphase, resuspended in Tris buffer (TBS, 20 mM Tris, 0.15 M NaCl, pH 7.4). After sedimentation by centrifugation at 250 x g for 10', the cells are washed and resuspended in TBS buffer and ready for use in experiments.

4. Adhesion study

The method of Berliner et al. (28) will be used. HUVEC are cultured in 6-well culture plates. At confluence, HUVEC monolayers are rinsed with serum-free medium for 3 times. Mononuclear cells from the patients or normal controls, $2x10^5$ cells in 250 µl in DMEM containing 1% heat-inactivated serum, are added to each well, and then kept in incubator. After 1 h, the nonadherent mononuclear cells are rinsed off and the wells fixed with 1% glutaraldehyde. The attached cells are counted in each of 10 microscopic fields.

Results

1. Study Subjects

A total of 59 cases of patients initially with low HDL-C (<40 mg/dl) were studied. They were studied before interventions and then after interventions to elevate the HDL-C level. All patients were studied at least 2 times on cell adhesion measurements and lipid profile at intervals of 3 months. There were 42 males and 17 females. The average age was 65.4±9.8 years for the study patients. The intervals between the studies of cell adhesion before and after intervention ranged between 26 days and 214 days, with an averaged of 88.4±15.5 days. Lipid lowering medicines included felofibrate in 45 patients, bezafibrate in 3 patients, lovastatin in 3 patients, gemfibrozil in 2 patients, simvastatin and fluvastatin each for 1 patient and 3 patients received combination therapy which included statin and fibrate.

2. Lipid levels before and after interventions

Table 1 showed the lipid data before and after interventions. Before intervention, the average cholesterol (CH) was 191.4±30.4 mg/dl which changed to 190.1±33.3 mg/dl (NS). The triglyceride levels changed significantly after interventions (210.2±115.8 mg/dl vs 151.3±96.6 mg/dl, $p<0.0001$). Low density lipoprotein cholesterol (LDL-C) showed no significant changes (118.3 ±27.1 mg/dl vs 121.6 ±28.6 mg/dl) while HDL-C revealed significant elevation (34.2 ±5.0 mg/dl vs 40.6±7.2 mg/dl, $p<0.0001$) after interventions.

Table 2 showed the lipid data of the 45 patients who received treatment with fenofibrate. Again the changes in CH and LDL-C were not significant but the changes in TG and HDL-C were highly significant ($p<0.0001$).

3. Cell adhesion before and after interventions

Cell adhesion for the whole group of patients averaged 144.2±50.0% before intervention which was changed to 115.5±30.5% after intervention (Table 1, $p<0.0001$). For the patients who were treated with felofibrate, the corresponding data were 138.3±48.1% vs 117.1±32.0% (Table, $p<0.01$).

Discussion

In this study, patients mainly with low HDL-C were studied for the adhesion of mononuclear cells to cultured HUVEC. As it has been definitely established that adhesion of mononuclear cells, especially monocytes, to the injured intima is the first and essential step to the development of atherosclerosis (1-4). Low in HDL-C as an important risk factor for atherosclerosis has been proved (22,23,29) and has been especially emphasized recently in a therapeutic guidelines (24).

In previous studies, we have proved that low HDL would increase the mononuclear cell adhesion to the cultured HUVEC. In this study we intended to further examine the effects of improvement in low HDL-C by various interventions on cell adhesions. In this study, we found that by using both life style modification and liid lowering medicines, TG was significantly decreased while HDL-C was markedly increased. The adhesion of mononuclear cell to the cultured HUVEC was demonstrated to significantly reduced after treatment with increased HDL-C and decreased TG. It is worth to note that in this study the CH and LDL-C were both kept stable after treatment. Therefore, the improvement of cell adhesion after treatment should attribute mainly to decreased TG and/or increased HDL-C.

The implications of this in vitro observation needs further long-term clinical study and observation to prove or disprove its value.

References

1. Ross R. The pathogenesis of atherosclerosis - An update. N Engl J Med 1986;314:488-500.
2. Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature 1993;362:801-9.
3. Schoen FJ, Cotran RS. Blood vessels. In Cotran RS, Kumar V, Collins T (eds): Pathologic Basis of Disease. W.B. Saunders, Philadelphia, 1999:507-509.
4. Gimbrone MA, Kume N, Cybulsky MI. Vascular endothelial dysfunction and the pathogenesis of atherosclerosis. In Weber PC, Leaf A (Eds): Atherosclerosis Reviews Vol. 25: Atherosclerosis: Cellular Interactions, Growth Factors, and Lipids. Raven Press, New York, 1993:1-9.
5. Kannel WB. An overview of the risk factors for cardiovascular disease. In Kaplan NM, Stamler J (Eds): Prevention of Coronary heart Disease, W.B. Saunders, Philadelphia, 1983:1-19.
6. Shen K, Sung KLP, Whittemore DE, DeLano FA, Zweifach BW, Schmid-Schoenbein GW. Properties of circulating leukocytes in spontaneously hypertensive rats. Biochem Cell Biol 1995;73:491-500.
7. Tropea BI, Huie P, Cooke JP, Tsao PS, Sibley RK, Zarins CK. Hypertension-enhanced monocyte adhesion in experimental atherosclerosis. J Vasc Surg 1996;23:596-605.
8. Liu Y, Liu T, McCarron RM, Spatz M, Feuerstein G, Hallenbeck JM, Siren A. Evidence for activation of endothelium and monocytes in hypertensive rats. Am J Physiol 1996; 270 (Heart Circ Physiol) 39:H2125-H2131.
9. Tsao PS, McEvoy LM, Drexler H, Butcher EC, Cooke JP. Enhanced endothelial adhesiveness in hypercholesterolemia is attenuated by L-arginine. Circulation 1994;89:2176-82.
10. Jongkind JF, Verkerk A, Hoogerbrugge N. Monocytes from patients with combined hypercholesterolemia-hypertriglyceridemia and isolated hypercholesterolemia show an increased adhesion to endothelial cells in vitro: II. Influence of intrinsic and extrinsic factors on monocyte binding. Metabolism 1995;44:374-8.
11. Weber C, Erl W, Weber KSC, Weber P. HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocyte to endothelium and reduce increased adhesiveness of monocytes isolated from patients with hypercholesterolemia. J Am Coll Cardiol 1997;30:1212-7.
12. Hackman A, Abe Y, Insull W, Pownall H, Smith L, Dunn K, Gotto A, Ballantyne CM. Levels of soluble cell adhesion molecules in patients with dyslipidemia. Circulation 1996;93:1334-8.
13. Davi G, Romano M,Mezzetti A, Procopio A, Iacobelli S, Antidormi T, Bucciarelli T, Alessandrini P, Cuccurullo F, Bon GB. Increased levels of soluble P-selectin in hypercholesterolemic patients. Circulation 1998;97:953-7.
14. Hoogerbrugge N, Postema PTE, Verkerk A, Jongkind JF, Jacobs ML. Hypertriglyceridemia enhances monocyte binding to endothelial cells in NIDDM. Diabetes Care 1996;19:1122-25.
15. Kim JA, Berliner JA, Natarajan RD, Nadler JL. Evidence that glucose increases monocyte binding to human aortic endothelial cells. Diabetes 1994;43:1103-7.
16. Menon RP, Sudhakaran PR. Enhanced adhesion of human mononuclear

cells to nonenzymatically glycosylated collagen I. Mol Cell Biochem 1995;148:115-21.
17. Haller U, Drab M, Luft FC. The role of hyperglycemia and hyperinsulinemia in the pathogenesis of diabetic angiopathy. Clin Nephrol 1996;46:246-55.
18. Delamaire M, Maugendre D, Moreno M, Le Goff MC, Allannic H, Genetet B. Impaired leukocyte functions in diabetic patients. Diabetic Medicine 1997;14:29-34.
19. Carantoni M, Reaven GM, Abbasi F, Tsao PS, Chu L, Varasteh B, Chen YDI, Cooke JP. Adherence of mononuclear cells to endothelium in vitro is increased in patients with NIDDM. Diabetes Care 1997;20:1462-5.
20. Desfaits AC, Serri O, Renier G. Normalization of plasma lipid peroxides, monocyte adhesion, and tumor necrosis factor-a production in NIDDM patients after gliclazide treatment. Diabetes Care 1998;21:487-93.
21. Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, Figliuzzi M, Remuzzi A, Zoja C, Remuzzi G. Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. J Clin Invest 1998;101:1905-15.
22. Lien WP, Lai LP, Shyu KG, Huang JJ, Chen JJ, Lei MH, Cheng JJ, Huang PJ, Tsai KS. Low serum high-density lipoprotein cholesterol concentration is an important coronary risk factor in Chinese patients with low serum levels of total cholesterol and triglyceride. Am J Cardiol 1996;77:1112-5.
23. Lien WP, Lai LP, Chen JJ, Huang JJ,Lin JL, Lei MH, Huang PJ, Tsai KS. A retrospective hospital-based study of coronary risk factor in Taiwan. Analysis of patients with established diagnosis. Japanese Heart J 1998;31:435-44.
24. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
25. Jaffe EA, Nachman RL, Becker CG, Minick c. Culture of human endothelial cells derived from umbilical vein; identification by morphologic and immunologic criteria. J Clin Invest 1973;52:2745-56.
26. Van Hinsbergh VWM, Scheffer MA, Langeler EG: Macro- and microvascular endothelial cells from human tissues. In Piper HM (ed.): Cell Culture Techniques in Heart and Vessel Research. 1990:178-204, Springer-Verlag, Berlin.
27. Menon RP, Sudhakaran PR. Enhanced adhesion of human mononuclear cells to nonenzymatically glycosylated collagen I. Mol Cell Biochem 1995;148:115-21.
28. Berliner JA, Territo MC, Sevanian A, Ramin S, Kim JA, Bamshad B, Esterson M, Fogelman AM. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. J Clin Invest 1990;85:1260-6.
29. Rubins HB, Robins SJ, Collins D, et al. Gemfibrozil for the secondary prrevention of coronary heart disease in men with low levels of high density lipoprotein cholesterol. N Engl J Med 1999;341:410-8.

Table 1 Cell adhesion and lipid profile before and after treatment

	Before treatment	After treatment
CH	191.4±30.4	190.1±33.3
TG	210.2±15.8	151.3±96.6*
LDL-C	118.3±27.1	121.6±28.6
HDL-C	34.2±5.0	40.6±7.2*
Cell Adhesion (%)	144.2±50.0	115.5±30.5*

Abbreviations: CH=cholesterol. HDL-C=high density lipoprotein cholesterol, LDL-C= low density lipoprotein cholesterol, TG=triglyceride
*p<0.0001 as comparing with that before treatment

Table 2 Cell adhesion and lipid profile before and after treatment in patients treated with felofibrate

	Before treatment	After treatment
CH	192.4±27.8	189.2±33.6
TG	198.6±19.9	143.5±101.9*
LDL-C	121.6±23.9	122.3±29.5
HDL-C	33.8±4.8	41.4±5.2*
Cell Adhesion (%)	138.3±48.1	117.1±32.0#

Abbreviation as in Table 1
*p<0.0001 as comparing with that before treatment
#p<0.01 as comparing with that before treatment

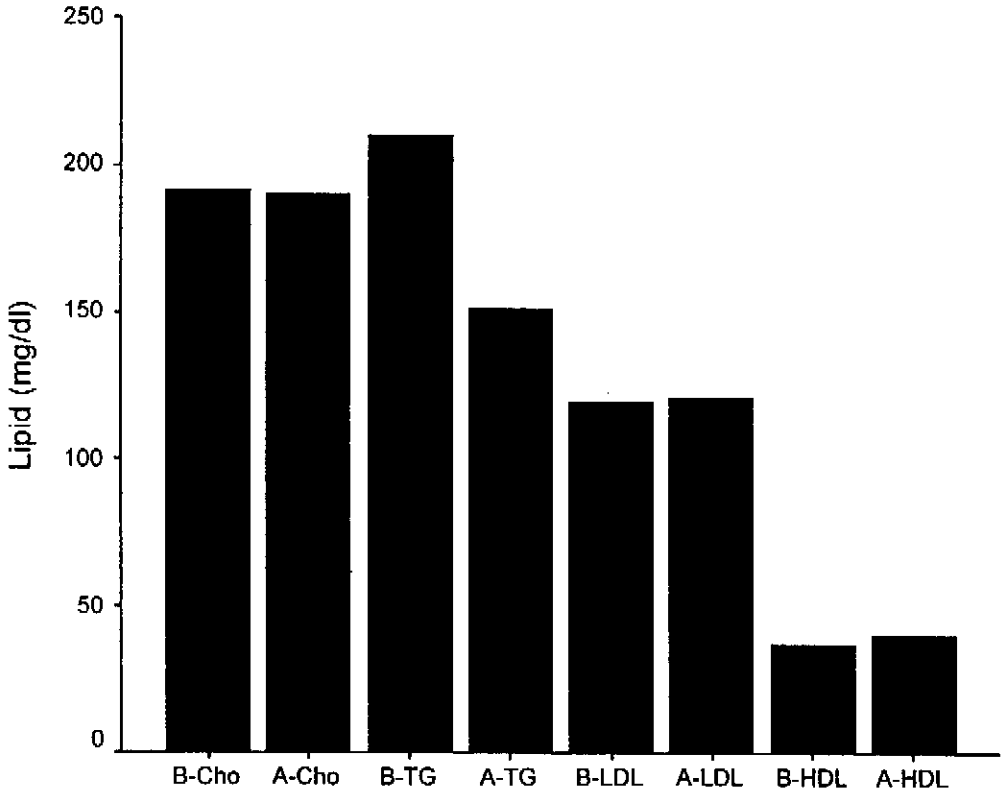

Fig. 1 Serum lipids in patients who were trearted with lipid-lowering agents

Abbreviations: A- = after treatment; B- = before treatment; Cho = cholesterol; HDL = high density lipoprotein cholesterol; LDL = low density lipoprotein Cholesterol; TG = triglyceride.

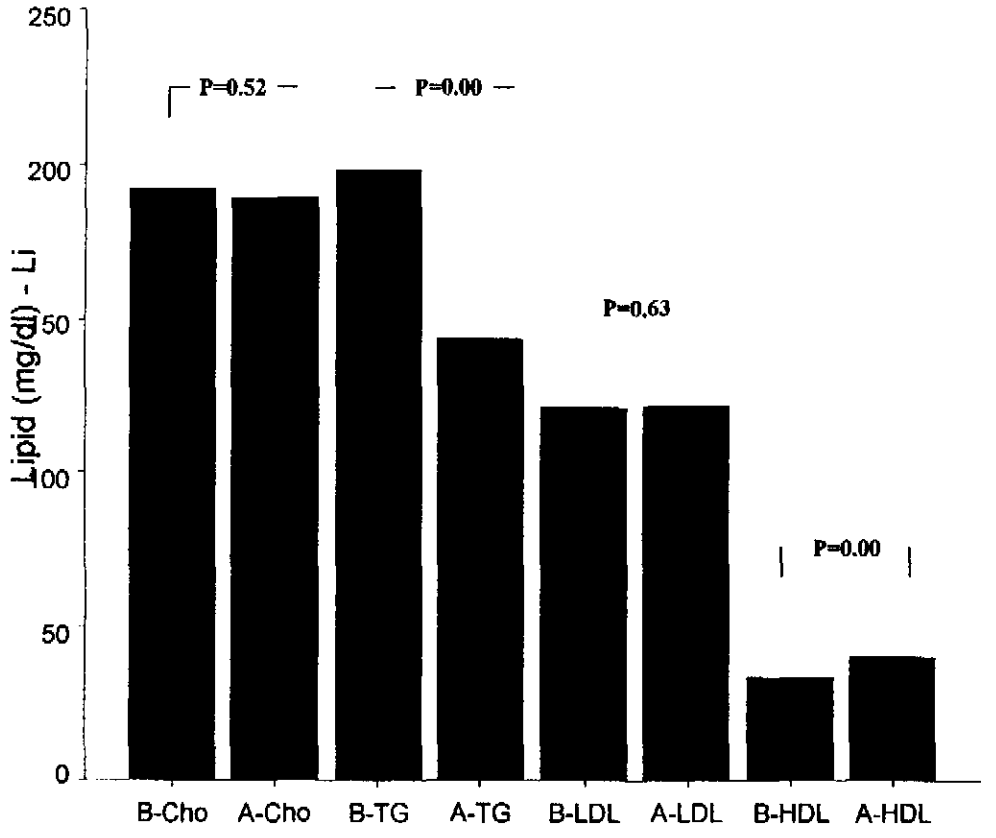

Fig. 2 Serum lipids in patients who were trearted with fenofibrate (Lipanthyl)
Abbreviations: A- = after treatment; B- = before treatment; Cho = cholesterol; HDL = high density lipoprotein cholesterol; LDL = low density lipoprotein Cholesterol; TG = triglyceride.