Potent Capillary Isotachophoresis (cITP) for Analyzing a Marker of Coronary Heart Disease Risk and Electronegative Low-Density Lipoprotein (LDL) in Small Dense LDL Fraction

Keita Noda, MD; Bo Zhang, PhD; Yoshinari Uehara, MD; Shin-ichiro Miura, MD; Akira Matsunaga, MD; Keijiro Saku, MD

Background The potency and usefulness of capillary isotachophoresis (cITP) for assessing whole-serum lipoprotein profiles and quantifying electronegative low-density lipoprotein (LDL) has been previously reported. Methods and Results A new cITP method to measure electronegative LDL in the small dense LDL fraction has been established. Both electronegative LDL and electronegative LDL in the small dense LDL fraction decreased after treatment with fenofibrate. Conclusions This method appears to be useful for analyzing a marker of coronary heart disease risk and may be suitable for evaluating the effects of hypolipidemic agents. (Circ J 2005; 69: 1568–1570) Key Words: Capillary isotachophoresis (cITP); Electronegative low-density lipoprotein (LDL); Small dense LDL

Capillary isotachophoresis (cITP) is a newly established technique for characterizing plasma lipoprotein subfractions according to their electric charges. It separates plasma lipoproteins into 8 fractions, comprising 3 high-density lipoprotein (HDL) fractions with fast (f), intermediate (I), and slow (s) electric mobility, a chylomicron/remnants fraction (fast very low-density lipoprotein (VLDL)), a VLDL/intermediate density lipoprotein (IDL) fraction (slow VLDL), 2 LDL fractions with fast and slow electric mobility, and a minor LDL fraction.1–5 In our previous reports, the fast LDL fractions corresponded to electronegative LDL in which some oxidized LDL, VLDL, and small dense LDL etc, the so-called “bad cholesterol”, are concentrated.4 After the light LDL fraction is precipitated from whole serum using heparin-Mg2+, electronegative LDL in the small dense LDL fraction can be measured using cITP.4 In the present study we used this method before and after treatment with fenofibrate.

Methods

Quantification of Lipoprotein Subfractions by cITP

cITP of serum lipoproteins was performed on a Beckman P/ACE MDQ system (Beckman-Coulter Inc, Tokyo, Japan) according to the method of Bottcher et al6 with some modifications. All of the reagents used in the cITP analysis were purchased from Aldrich-Sigma (Tokyo, Japan) unless indicated otherwise. Dimethylpolysiloxane modified fused silica capillary (AT™-1) was purchased from Alltech Japan Inc (Tokyo, Japan). A 6 μl sample of serum was diluted with 14 μl of leading buffer consisting of 10 mmol/L HCl and 18 mmol/L ammediol (2-amino-2-methyl-1,3-propanediol) (pH 8.8), prestained with 10 μl 0.1 mg/ml NBD C6-ceramide (Molecular Probes, Inc, OR, USA) for 5 min at room temperature, and mixed with 50 μl of a mixture containing leading buffer with 0.35% hydroxypropylmethylcellulose, spacers, and 5-carboxy-fluorescein as an internal marker. The spacers included were the same as those described by Bottcher et al, including N-(2-acetamido)-2-aminoethanesulfonic acid, D-glucuronic acid, 1-octanesulfonic acid sodium salt, 3-(N-tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid, L-serine, L-glutamine, L-methionine, and glycine. The terminating buffer contained 24 mmol/L l-alanine and 13 mmol/L ammediol, and was adjusted to pH 10.5 with saturated barium hydroxide solution. The sample was injected for 18 s at 20 pounds into a 30 cm-long capillary (inner diameter 180 μm), and separation was performed at a constant 30 mA for 1 min and 10 kV for 7 min. The separated zones were monitored with argon-laser-induced fluorescence detection (excitation, 488 nm; emission, 520 nm). Each peak was identified and the peak area in relative fluorescence units was analyzed using 32 Karat Software version 5.0 (Beckman-Coulter Inc). The capillary was washed with leading buffer and water between runs.

Separation of Light and Dense LDL Subfractions by Heparin-Mg2+ Precipitation

The light LDL subclass was separated from the dense LDL subclass using the heparin-Mg2+ precipitation method described by Hirano et al7 with some modification as described previously.2
Results and Discussion

Fig 1a,b shows the whole-serum lipoprotein profiles by cITP in a patient with type IV hyperlipoproteinemia with HDL deficiency (HDL-cholesterol 38 mg/dl) before and after treatment with fenofibrate. Increased fast VLDL (peak 4), slow VLDL (peak 5) and fast LDL (peak 6), and decreased slow LDL (peak 7) were observed before drug treatment (Fig 1a), and peaks 4, 5 and 6 clearly decreased after treatment (micronized fenofibrate 100 mg, bd for 2 months) (Fig 1b). Whole-serum lipid profiles were depleted of large light LDL (which is the small dense LDL fraction) before (Fig 1c) and after (Fig 1d) treatment with fenofibrate. The serum depleted of large light LDL fractions of fast VLDL (peak 4), slow VLDL (peak 5), fast LDL (peak 6), and slow LDL (peak 7) before fenofibrate (Fig 1c) was similar to the whole-serum lipid profile before treatment (Fig 1a), which means that most of the apo B fraction was in the small LDL dense fraction in this case, and indicates that the precipitation of light LDL does not affect the content of the HDL fractions. However, after treatment with fenofibrate, most of the fast VLDL (peak 4), slow VLDL (peak 5), and fast LDL (peak 6) fractions decreased without affecting the HDL and slow LDL (peak 7) fractions (Fig 1d). Compared with the whole-serum lipid profile before treatment (Fig 1b), the most striking reduction was observed in peak 6 (Fig 1d). Serum triglycerides, HDL-cholesterol and LDL-cholesterol were, respectively, 233, 38 and 69 mg/dl before treatment, and 85, 42, and 120 mg/dl after treatment. Similar results were observed in other patients who were treated with fenofibrate. An advantage of this technique is that we can quantitatively measure electronegative LDL, a potent marker of coronary heart disease risk, in serum depleted of large light LDL (ie, the small dense LDL fraction). Previous studies have shown that electronegative LDL is the most atherogenic component and we previously reported that electronegative LDL is associated with insulin resistance and carotid artery atherosclerosis as determined by ultrasonography. Therefore, a large trial will be needed to confirm that this fraction is an extremely bad lipoprotein fraction.

Acknowledgments

This work was supported by grants-in-aid from the Ministry of Education, Science and Culture of Japan (No.15790403), and by research grants from the Central Research Institute of Fukuoka University.

References

Correlation of high density lipoprotein (HDL)-associated sphingosine 1-phosphate with serum levels of HDL-cholesterol and apolipoproteins. *Atherosclerosis* 2005; 178: 199–205.


