Heterogeneity of Lipoprotein Electrophoretic Patterns in Patients with Type IIa Hyperlipoproteinemia

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Previous evaluations of plasma lipoproteins from patients with type IIa hyperlipoproteinemia have demonstrated quantitative but not qualitative differences from lipoproteins isolated from normal subjects. Using a reproducible, sensitive, high-resolution agarose gel electrophoretic system, we evaluated the plasma lipoprotein of 22 consecutive, unrelated patients with type IIa hyperlipoproteinemia. Five distinct electrophoretic patterns were observed. This electrophoretic heterogeneity in type IIa hyperlipoproteinemia reflects subtle charge differences in the lipoproteins from these patients, which were not detected with paper electrophoresis or with lipoprotein quantification. There was little, if any, difference in total plasma cholesterol, low- or very-low-density lipoproteins, plasma triglycerides, or concentrations of apolipoproteins A-I, A-II, B, C-II, or E in these subgroups. However, unique clinical features were correlated with the electrophoretic patterns. These findings support the concept that the type IIa hyperlipoproteinemic phenotype includes several molecular defects that are reflected in subtle differences in lipoprotein charge.

Additional Keyphrases: apolipoproteins · electrophoresis, agarose gel · variation, source of differences in lipoprotein charge · molecular defects

The primary hyperlipoproteinemias can be divided into five distinct lipoprotein phenotypes (1), based on the quantification of individual lipoprotein fractions and the results of paper electrophoresis. Each of these phenotypes may include one or more molecular defects (2). Type IIa hyperlipoproteinemia, characterized by an increased concentration of low-density lipoprotein (LDL) cholesterol, has been demonstrated to include patients with at least two different genotypes. One of these, familial hypercholesterolemia (FH), has been demonstrated to be due to a defect in cell surface–LDL interaction (3). The type IIa phenotype is also observed in patients with familial combined hyperlipidemia (4). These patients, however, do not have the same molecular defect as occurs in FH. Thus, the type IIa phenotype appears to include at least two different underlying etiologies. Abnormalities in lipoproteins or apolipoproteins could also result in the IIa phenotype. By using a sensitive, high-resolution electrophoretic system, one can evaluate subtle qualitative differences in plasma lipoproteins. We used agarose gel electrophoresis to evaluate the plasma from 22 consecutive, unrelated patients who had a IIa phenotype and observed five distinctly different electrophoretic patterns. This electrophoretic heterogeneity may reflect different underlying molecular defects, all of which result in the type IIa hyperlipoproteinemia phenotype.

Materials and Methods

All study subjects were outpatients of the Molecular Disease Branch, whose cases were followed at the Clinical Center, National Institutes of Health. Consecutive patients willing to discontinue drug medication were enrolled in the study. These individuals, who were unrelated, on an ad lib diet, and within 10% of ideal body weight, had normal thyroid, renal, and hepatic function as well as normal electrophoretic profiles of serum proteins. All lipid-lowering agents were discontinued at least four weeks before the quantification and electrophoresis of the lipids and lipoproteins. Histories were taken and physical examinations conducted by physicians in the Molecular Disease Branch. The presence of peripheral vascular disease was defined as symptomatic claudication. Coronary artery disease was designated as present if the patient had angina, myocardial infarction, or angiographically documented disease. Cerebrovascular disease was considered present if the patient had a history of a stroke or transient ischemic attack.

Blood collected from subjects after a 12- to 14-h overnight fast was mixed with EDTA (1 g/L), and the plasma was separated at 4 °C in a refrigerated centrifuge. Plasma cholesterol and triglycerides were quantified by an enzymic method with a Gilford 3500 (Gilford Instrument Labs., Oberlin, OH 44074). HDL cholesterol in the plasma was determined after dextran sulfate precipitation (5). Plasma was ultracentrifuged (d = 1.006) for 18 h at 39 000 rpm (4 °C) in Beckman 40.3 rotors (Beckman Instruments, Fullerton, CA 92634) (6), and the VLDL was separated from the other plasma lipoproteins by tube slicing (6). The cholesterol concentration in the d < 1.006 infranate was measured, and the concentrations of VLDL and LDL cholesterol were calculated (7). All study subjects had plasma cholesterol and LDL cholesterol concentrations two standard deviations or more above the normal mean, and VLDL cholesterol and total plasma triglyceride concentrations within two standard deviations of the normal mean, consistent with type IIa hyperlipoproteinemia (1, 2).

Plasma concentrations of apolipoproteins A-I, A-II, B, and C-II were determined by radial immunodiffusion against antiserum from goats, sheep, or rabbits (8); apoE was determined by a double-antibody radioimmunoassay. Blood samples for the agarose gel electrophoresis were collected at the same time as for lipoprotein quantification. After the blood was allowed to clot at room temperature, we separated the lipoproteins by electrophoresis (at a constant 140 V, 12 min) within 6 h of drawing, using an agarose gel electrophoretic system as previously described (9).

Results

Agarose gel electrophoresis of serum from patients with type IIa hyperlipoproteinemia revealed five distinct patterns, based on band position and density (Figure 1). All samples demonstrated a dense β-lipoprotein band. Three
These had displayed a hyperlipoproteinemia. VLDL and HDL values, on the other hand, were normal. The concentrations of LDL, total cholesterol, and VLDL in the different subgroups were similar to the concentrations observed in the entire type IIa cohort. However, the HDL cholesterol in the slow-β group was higher than in the total group (p < .05).

The plasma apolipoprotein concentrations are shown in Table 2. The only difference between the entire group of type IIa subjects and normal control values was the striking increase in the concentration of apoB in the IIa patients. This nearly twofold increase in apoB concentration was significantly higher (p < .05 by Student's t-test). The concentrations of apolipoproteins A-I, A-II, C-II, and E were similar among the different subgroups. Except for the double pre-β subgroup, the slow pre-β subgroup had significantly higher apoB content than did the other subgroups (p < .05).

There appears to be an equal distribution of the sexes in the electrophoretic subgroups, but patients demonstrating a slow-β band were younger than the rest of the subgroups (31 ± 2.9 vs 47 ± 7.5 years). The only patient with symptoms of cardiovascular disease demonstrated a strong double-β band. She had had a right hemispheric cerebrovascular accident at age 57 and had residual left-sided hemiparesis and paresthesia. None of the patients with the fast pre-β band had corneal arcus. Vascular disease of one form or another and tendon xanthoma were present in each of the subgroups.

Discussion

Each of the primary hyperlipoproteinemia phenotypes contains a number of basic underlying molecular defects (2). The possibility that type IIa hyperlipoproteinemia may be due to an abnormality in LDL has been previously evaluated. Except for slight differences in triglyceride concentration, LDL from type IIa patients is similar to LDL from normal subjects (10, 11). Although the physical properties of apoB have limited full evaluation of this apolipoprotein, the amino acid composition and antigenic properties of apoB from type IIa subjects parallel closely those of normal apoB (12, 13). The metabolism of LDL from FH homozygotes, injected into a normal subject, was normal (14). In addition, LDL from a FH homozygote bound and suppressed hydroxymethylglutaryl-CoA reductase in cultured fibroblasts similar to normal LDL (15). Therefore, previous studies have not disclosed abnormalities in LDL from type IIa subjects. However, these studies have been limited to LDL from patients with FH.

Recently, an abnormality or absence of an apolipoprotein has been demonstrated to lead to a dyslipidemic phenotype. By sodium dodecyl sulfate–gel electrophoresis as well as isoelectric focusing, the apoE from type III patients is subtly but distinctly abnormal (16, 17). The apoE isolated from patients with type III hyperlipoproteinemia was metabolized more slowly than normal apoE in both normal and type III subjects (18). Furthermore, the absence of apoE has also been shown to result in a type III lipoprotein phenotype (19). Thus, lipoprotein phenotypes can result from an abnormality or an absence of an apolipoprotein that can only be delineated by sensitive, high-resolution techniques.

By using the sensitive agarose gel electrophoretic system, we found that the 22 unrelated type IIa hyperlipoproteinemic patients in the present study had five distinctly differ-
ent patterns, but this qualitative heterogeneity in the lipoproteins was not correlated with any quantitative changes in the lipid composition of the lipoprotein subfractions. This charge difference could be caused by subtle differences in amino acid composition of the apoprotein, which have not been previously noted because earlier studies involved LDL from FH patients. Alternatively, other polar moieties such as phospholipids or carbohydrates could differ in these subgroups. Indeed, the carbohydrate composition in LDL from type IIA patients reported differs from normal LDL (20). Finally, the charge heterogeneity of these subgroups may reflect a change in the distribution of the apolipoproteins on the different lipoprotein particles. Although these different patterns may reflect a secondary rather than causal role in type IIA hyperlipoproteinemia, they may point to genetic heterogeneity within this lipoprotein phenotype. Further genetic studies with this electrophoretic technique as well as more extensive analysis of the concentration and distribution of the charged moieties in these IIA subgroups are warranted.

### References


