case reports

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An Alpha Slow-Moving High-Density-Lipoprotein Subfraction in Serum of a Patient with Radiation Enteritis and Peritoneal Carcinosis

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An alpha slow-moving high-density-lipoprotein (HDL) subfraction was seen in a patient presenting with radiation enteritis and peritoneal carcinoma, who was given long-term cyclic parenteral nutrition. This subfraction, observed in addition to normal HDL, was precipitated with low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL) by sodium phosphotungstate–magnesium chloride. The patient’s serum lipoproteins were analyzed after fractionation by density gradient ultracentrifugation. The alpha slow-moving HDL fraction floated in the ultracentrifugation subfractions with densities ranging from 1.028 to 1.084 kg/L, and their main apolipoproteins included apolipoprotein E in addition to apolipoprotein A-I. These HDL were larger than HDL-2. The pathogenesis of this unusual HDL subfraction is hypothesized.

Additional Keyphrases: apolipoprotein E-containing HDL · cholesteryl ester transfer protein

An alpha slow-moving HDL subfraction is rarely seen on a lipoproteinogram.3 So far, it has only been observed during particular events in subjects with chronic cholestasis. Thus, various authors have reported its presence in the early stages of primary biliary cirrhosis (1, 2) and in mild intrabiliary cholestasis (3), and we ourselves described it during periods of moderate icterus in children with chronic cholestasis from birth and severe hypercholesterolemia (4). We subsequently demonstrated that this HDL subfraction was rich in apolipoprotein E (apo E) and was precipitated by sodium phosphotungstate–magnesium chloride (4). Here, we report the occurrence of an alpha slow-moving HDL subfraction that displayed similar properties, seen in the serum of a patient who until then had a very low cholesterol concentration, reflecting malnutrition resulting from intestinal failure, and who developed peritoneal carcinoma.

Case Report

The patient, a 51-year-old man, presented with an occlusive syndrome caused by radiation enteritis after radiotherapy for testicular carcinoma. He was hospitalized in December 1987 and given total cyclic parenteral nutrition. His nutrition included 20% Intralipid® (from 1700 to 2000 h) and a mixture of amino acids (Vamin®), glucose, electrolytes, vitamins, and trace metals (from 2000 to 0800 h).

During the three months after admission, a severe inflammatory syndrome persisted; C-reactive protein concentrations ranged from 50 to 90 mg/L (upper usual value: 5 mg/L) and α1-antitrypsin concentrations from 5.20 to 7.50 g/L (usual values: 2 to 4 g/L). No improvement in the patient’s nutritional status was observed during this period. His body weight never exceeded 44 kg and his serum albumin, transhylase, and retinol-binding protein concentrations remained: 18 to 27 g/L, 80 to 200 mg/L, and 37 to 56 mg/L, respectively. Results of liver-function tests were within the normal reference interval until the end of January 1988. In February, however, the patient’s alkaline phosphatase (EC 3.1.3.1) and gamma-glutamyltransferase (EC 2.3.2.2) activities were high; on February 8 they reached 278 U/L (upper normal value: 90 U/L) and 107 U/L (upper normal value: 40 U/L), respectively. Subsequently, both decreased. Bilirubin concentrations consistently remained within normal limits.

On March 28, surgery to excise the lesioned parts of the small intestine revealed that complete peritoneal carcinoma had invaded the intestine, liver, and pancreas. The patient died 48 h later.

Materials and Methods

During the patient’s three-month hospitalization, seven blood samples were collected at various times, into Vacutainer Tubes (Becton-Dickinson, 38035 Grenoble, France), at 0800 h—i.e., 12 h after the end of a session of Intralipid infusion.

The concentrations of total cholesterol, triglycerides, and phospholipids in the serum were determined enzymatically in a Hitachi 737 analyzer (Boehringer Mannheim, 38240 Meylan, France) with reagents from Boehringer. The reagent for nonesterified cholesterol was from Biotrol, 75140 Paris Cedex 03, France. The total lipid concentrations in the serum were calculated (sum of the different serum lipid components expressed in grams per liter; the concentration of cholesteryl esters was estimated as the concentration of esterified cholesterol × 1.7).

High-density lipoproteins, cholesterol, and HDL phospholipids were measured as previously described (4). Serum apolipoproteins A-I and B (apo A-I and apo B) were determined by laser immunonephelometry, with use of reagents from Behring-Hoechst, 92304 Rueil-Malmaison, France. Lipoproteinograms were made on polyacrylamide–agarose plates with a discontinuous acrylamide gradient (20 and 30 g/L gels; Lipofilm, Sebia, 92130 Issy les Moulineaux,
France). Lipoprotein X was studied according to Seidel et al. (5). The alpha slow-moving HDL were precipitated from the serum by the method we previously described (4). The concentrations of the alpha slow-moving and "normal" HDL in serum were expressed as grams of lipids per liter of serum.

We performed density gradient ultracentrifugation of lipoproteins with densities of 1.006 to 1.25 kg/L at 40 000 rpm and 4 °C for 48 h in an SW 41 rotor in a Beckman-Spinco L 2 ultracentrifuge, using a modified version of the method of Redgrave et al. (6). The contents of the ultracentrifugation tubes were collected as seven 1.5-mL aliquots, which were dialyzed against phosphate buffer (10 mmol/L, pH 7.4). The lipid components of the aliquots were measured as above for serum lipids. Proteins were determined by the method of Lowry et al. (7), with bovine serum albumin as standard. Apolipoprotein distribution was studied by sodium dodecyl sulfate–gradient polyacrylamide gel electrophoresis (SDS-PAGE, 30–200 g of acrylamide per liter). The molecular-mass calibration kit was the "Low Molecular Weight" kit (Pharmacia, 78390 Bois d'Arcy, France). The size of the lipoproteins was measured by polyacrylamide gradient gel electrophoresis (30–200 g of acrylamide per liter). Gels were scanned with a Sebia Cellosystem apparatus and calibrated for particle radius with use of the "High Molecular Weight" electrophoresis kit from Pharmacia.

**Results**

The serum lipid components of the patient, determined during his hospitalization (Table 1), showed cholesterol always to be subnormal, except in the samples collected on March 2 and 14, which respectively displayed normal and moderately subnormal values. Total-lipid concentrations in these two last samples were 7.30 and 5.15 g/L. The concentrations of HDL cholesterol, HDL phospholipids, and apo A-I—at first normal or slightly subnormal—increased to the upper limit of normal on March 2 and then declined sharply on March 14. Note that the values found for HDL cholesterol and phospholipids were those of "normal" HDL, because the alpha slow-moving HDL are precipitated by sodium phosphotungstate–MgCl₂. On these dates, a decreased apo B was also measured, suggesting a diminution in LDL. Furthermore, the lipoproteinograms (Figure 1) revealed the presence of an additional lipoprotein fraction characterized by its alpha slow electrophoretic migration. We did not detect any lipoprotein X in the samples collected on March 2 and 14.

The concentrations of the alpha slow-moving HDL and "normal" HDL in the serum samples collected on these two dates were, expressed as lipids, 2.60 and 1.80 g/L (March 2) and 2.10 and 1.10 g/L (March 14). Therefore, in the March 2 and 14 serum samples the alpha slow-moving HDL carried 35.6% and 42.8% of the serum total lipids in serum, i.e., more than the patient's normal HDL, which carried 24.6% and 22.4%, respectively.

These unusual HDL subfractions contained relatively more cholesterol, especially nonesterified cholesterol, than did the "normal" HDL (in percentages, nonesterified cholesterol constituted 15.1% on March 2 and 14.7% on March 14 vs 4.8% and 5.0%, cholesterol esters 32.7% and 31.9% vs 30.4% and 31.8%, triglycerides 4.4% and 3.8% vs 7.1% and 11.2%, and phospholipids 47.8% and 49.5% vs 57.7% and 52.0%).

Together, these data explain the increase in serum nonesterified cholesterol that we observed on these two dates and the surprisingly normal value for total cholesterol in the March 2 sample, despite the decrease in LDL.

The lipoproteins of the sample collected on March 2 were evaluated after density gradient ultracentrifugation. As shown by the lipoproteinograms of the different subfractions in Figure 2, the alpha slow-moving HDL could not be completely separated from the other lipoprotein classes by ultracentrifugation. They floated in ultracentrifugation subfractions 3 and 4 (density ranges: 1.028 to 1.050 and 1.050 to 1.084 kg/L). Subfraction 3 also contained LDL, and subfraction 4, "normal" HDL, which certainly included HLD₁ and some HLD₂. Consequently, the composition of these subfractions reflects the presence of different lipoprotein populations (Table 2). Subfractions 5 and 6 had densities and compositions enabling us to identify them as HLD₂ and HLD₅, respectively.

The apolipoprotein patterns of the ultracentrifugation subfractions indicate that subfraction 3 contained apo B supplied by the LDL, as well as large amounts of apo E (Mr = 35 000) and apo A-I (Mr = 28 500) supplied by the alpha slow-moving lipoproteins (Figure 3). Furthermore, SDS-PAGE indicated that subfraction 4 was richer in apo E than HLD₂ are normally, so this excess of apo E was certainly supplied by the alpha slow-moving HDL.

This unusual lipid and apolipoprotein composition was combined with changes in HDL particle size. Very large particles with radii of more than 6.1 nm were present in subfraction 3, which contained the alpha slow-moving HDL and was richest in apo E (Figure 4). The HDL particles in subfraction 4 were polydisperse, and those with the largest radii certainly constituted alpha slow-moving HDL. The scans of the two other subfractions (numbers 5 and 6) showed that their radii, ranging from 3.5 to 5.2 nm, corresponded to those of normal HDL.

<table>
<thead>
<tr>
<th>Date of study (1988)</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Apolipoprotein A-I</th>
<th>Apolipoprotein B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.9–6.5</td>
<td>1.3–2.1</td>
<td>0.5–1.3</td>
<td>2.1–3.1</td>
<td>0.9–1.6</td>
</tr>
<tr>
<td>Nonesterified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01/25</td>
<td>3.1</td>
<td>0.91</td>
<td>1.7</td>
<td>2.4</td>
<td>1.10</td>
</tr>
<tr>
<td>01/11</td>
<td>2.8</td>
<td>0.78</td>
<td>1.4</td>
<td>2.1</td>
<td>1.05</td>
</tr>
<tr>
<td>01/15</td>
<td>2.8</td>
<td>0.78</td>
<td>1.8</td>
<td>2.3</td>
<td>0.80</td>
</tr>
<tr>
<td>02/04</td>
<td>2.1</td>
<td>ND</td>
<td>0.9</td>
<td>1.7</td>
<td>ND</td>
</tr>
<tr>
<td>02/12</td>
<td>2.6</td>
<td>ND</td>
<td>1.0</td>
<td>2.6</td>
<td>ND</td>
</tr>
<tr>
<td>03/02</td>
<td>5.8</td>
<td>1.85</td>
<td>0.7</td>
<td>3.9</td>
<td>1.6</td>
</tr>
<tr>
<td>03/14</td>
<td>3.4</td>
<td>1.20</td>
<td>0.8</td>
<td>2.9</td>
<td>0.55</td>
</tr>
</tbody>
</table>

ND: not determined.

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Table 2. Composition and Concentration of Lipoproteins in Gradient Ultracentrifugation Subfractions in the Serum Sample Collected on March 2, 1988

<table>
<thead>
<tr>
<th>Subfractions</th>
<th>Density range, kg/L</th>
<th>Concentration, g/L</th>
<th>Nonesterified cholesterol</th>
<th>Cholesteryl esters*</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.006–1.020</td>
<td>0.34</td>
<td>6.1</td>
<td>25.9</td>
<td>27.5</td>
<td>26.5</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>1.020–1.028</td>
<td>1.35</td>
<td>6.7</td>
<td>28.1</td>
<td>14.8</td>
<td>25.2</td>
<td>25.2</td>
</tr>
<tr>
<td>3</td>
<td>1.028–1.050</td>
<td>1.95</td>
<td>9.3</td>
<td>29.2</td>
<td>6.6</td>
<td>26.7</td>
<td>28.7</td>
</tr>
<tr>
<td>4</td>
<td>1.050–1.084</td>
<td>2.86</td>
<td>9.5</td>
<td>20.6</td>
<td>2.8</td>
<td>30.1</td>
<td>37</td>
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<tr>
<td>5</td>
<td>1.084–1.13</td>
<td>2.13</td>
<td>3.3</td>
<td>20.6</td>
<td>2.4</td>
<td>30</td>
<td>43.7</td>
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<tr>
<td>6</td>
<td>1.13–1.18</td>
<td>0.74</td>
<td>2.9</td>
<td>18.7</td>
<td>6.8</td>
<td>21.6</td>
<td>50</td>
</tr>
</tbody>
</table>

*Estimated as the concentration of esterified cholesterol x 1.7.

Discussion

We have described here an unusual alpha slow-moving HDL subfraction in the serum of a patient who had radiation enteritis, extensive peritoneal carcinosis, and persistent under-nutrition despite total parenteral nutrition. This HDL subfraction was characterized by its alpha slow electrophoretic migration and its high apo E content. Its constituent lipoproteins were larger than normal HDL. On ultracentrifugation, the alpha slow-moving HDL floated not only in the normal HDL density range but also in the LDL range. The higher their apo E content, the lower their density and the larger their size. These HDL closely resembled those we described earlier in serum from children presenting with chronic cholestasis from birth (4), although in the earlier work they were only found in the 1.063–1.21 kg/L fraction of sequential ultracentrifugation. They seemed very similar to the HDLc described by Mahley et al., cited in this earlier work (4). These HDLc are presumed to have a lipid-transport function, especially a role in reverse cholesterol transport (8).

Note that, in our patient, the only normal values for total and nonesterified cholesterol were not attributable to an improvement of his nutritional status, as his serum lipid profile might suggest, but to the presence of the alpha slow-moving HDL. Neither were they attributable to the presence of lipoprotein X, which is rich in nonesterified cholesterol and may be formed in serum during parenteral nutrition with Intralipid (9, 10).

As already stressed, alpha slow-moving HDL have always been observed in patients with mild cholestasis or moderate icterus during the chronic cholestasis syndrome. We previously suggested (4) that the main function of the apo E-rich HDLc sometimes seen in the serum of children with chronic cholestasis might be to help eliminate excess peripheral cholesterol.

The patient in the present study displayed two original features. First, to our knowledge, he never had hypercholesterolemia during the first two months in the hospital, i.e., before the appearance of the alpha slow-moving HDL. Secondly, his highest values for gamma-glutamyltransferase, observed on February 12 and 25 (88 and 107 U/L, respectively), were much lower than those of the cholesstatic children in our previous study, whose mean value was 725 U/L and his moderate cholestasis episode was anicteric. Although the serum lipid profile was not investigated on February 25, the relative enhancement of cholesterol and the alpha slow-moving HDL apparently occurred once cholestasis had regressed (alkaline phosphatase activities: 163, 131, and 123 U/L on March 2, 9, and 16, respectively). Consequently, the occurrence of the alpha slow-moving HDL is unlikely to reflect the need for reverse transport to the liver of a great excess of cholesterol. Weisgraber (cited in 11) reported that the formation in human plasma of HDL containing only apo E appears to be inversely proportional to the cholesteryl ester transfer activity (11). The accumulation of the alpha slow-moving HDL in the serum of our patient might therefore reflect a disorder affecting this activity. The latter is caused by the presence of a protein (12, 13) that is secreted by macrophages (14) or hepatocytes (15) and promotes the transfer of cholesteryl esters from HDL to lipoproteins with lower densities, and also into certain cells (15). Although we cannot produce any evidence for this hypothesis, we suggest that, in our patient, a deficiency of
lipid transfer activity might have been involved in the
accumulation of alpha slow-moving HDL. This deficiency
might have been the result of either reduced secretion of
the cholesteryl ester transfer protein or the presence in the
serum of an inhibitor of this protein (17), both alternatives
being connected with the extension of peritoneal carcinosis.

The skillful technical assistance of Nicole Marcardon is gratefully
acknowledged.

Fig. 3. SDS-PAGE electrophoretogram (50–200 g/L gradient) of apolipoproteins in the subtraction yields by gradient density ultracentrifugation and treated with β-mercaptoethanol (sample collected on March 2, 1985)

Protein molecular mass standard (S) was “Low Molecular Weight” (Pharmacia) and contained phosphorylase B (94,000), serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and lactalbumin (14,400). For density ranges of subfractions, see legend to Fig. 2.

References
13. Cheung MC, Wolf AC, Lum K, Tollefson JH, Albers JJ.

Fig. 4. Scans of particle size distribution of the lipoproteins in the subfraction yields by gradient density ultracentrifugation

Lipoproteins underwent electrophoresis on 30–200 g/L polyacrylamide gel and were stained with Coomassie Blue G 250. For density ranges of subfractions, see legend to Fig. 2. Particle radius, in nm, of proteins used as standards (“High Molecular Weight,” Pharmacia): thyroglobulin (8,50), ferritin (8,10), catalase (5,2), lactate dehydrogenase (4,06), and albumin (3,55).


