Hyperlipidemia in Acute Lymphoblastic Leukemia

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ABSTRACT

Studies were conducted on lipemic serum obtained from a 26 month old male to determine possible mechanisms for the association of a Type V hyperlipidemic phenotype with advanced lymphoblastic leukemia (ALL). Antibodies to apolipoproteins and endogenous heparin were not detected as previously reported. Fatty acid analysis of the triglyceride esters revealed a high proportion of stearic-acid (18:0) which was associated with a slower in vitro degradation of very low density lipoproteins (VLDL) by human milk lipoprotein lipase (LPL). This suggests that a cause of the hyperlipidemia could be abnormal composition of triglycerides which render the VLDL a poor substrate for lipoprotein lipase. Hyperlipidemia in leukemia may be more prevalent than previously realized since nine other cases of newly diagnosed ALL have been studied who had moderate hypertriglyceridemia associated with elevated ApoB and low ApoA-I levels, but normal triglyceride composition. These findings suggest that the abnormal triglyceride composition is a late feature of the hyperlipidemia in leukemia, as observed in the case studied.

Introduction

There is a known association of hyperlipidemia with cancer. Aberrations in lipid metabolism involving hepatic lipoprotein production or disposal are thought to play a role, but specific defects have not always been found. An exception to this is the finding that antibodies with antiheparin activity may inhibit lipoprotein lipase activity. Other possibilities are that antibodies against apolipoproteins may cause matting together of the triglyceride-rich lipoproteins so that they fail to activate lipoprotein lipase. The apoprotein activator of lipoprotein lipase, ApoC-II may then fail to be exposed to the enzyme or, alternatively, antibodies may be directed against ApoC-II or lipoprotein lipase. In addition, inhibitors of lipoprotein lipase may be present in excess such as ApoC-III or ApoE, or possibly unidentified inhibitors may exist which are also unique to the leukemic process.
Therefore, in view of the typical Type V phenotype of the plasma obtained from a newly diagnosed case of acute lymphoblastic leukemia, these possible mechanisms for the hyperlipidemia were explored.

Case Report

A 26 month old male was admitted to hospital with a diagnosis of advanced leukemia. The family history indicated neurofibromatosis in the mother and maternal grandmother, who had died of cancer at the age of 31 years. On admission, the patient was observed to be tachypneic and in severe respiratory distress. The height was 34 1/2 inches (75th percentile) and the weight was 27 lbs (25th percentile). The pulse rate was 160, and the respiratory rate 70 beats per minute. He was pale with petechiae on the trunk and legs and had multiple café au lait spots. Hepatosplenomegaly was detected. A chest x-ray revealed bilateral diffuse infiltrates and cardiomegaly. Initial laboratory tests confirmed the presence of acute lymphoblastic leukemia. The white blood count was 182.4 x 10^9 per L (blast cells 63 percent, neutrophils 15 percent, lymphocytes 13 percent), myelocyte:metamyelocyte ratio was 1:2, platelets 19 x 10^9 per L. The bone marrow showed a grade 1 cellularity, with 88 percent lymphoblasts, 6 percent myeloids, 1 percent erythroblasts, and 5 percent lymphoids. Chemistry: Sodium 137, potassium 6.2, chloride 109 mEq per l, CO₂ 6 mEq per l, BUN 38 mg per dl, creatinine 0.8 mg per dl, glucose 177 mg per dl, uric acid 16.2 mg per dl, SGOT 63 IU per l, and amylase 9 IU per l. The hematocrit was only 5 percent and required a partial exchange transfusion with 250 cc packed red blood cells. The serum obtained was noted to be lipemic. The post exchange hematocrit was observed to be 24 percent, after which 250 cc of platelet concentrate were infused. Subsequently, the urine output began to decrease over a four hour period. The heart rate slowed down to 42 per minute followed by respiratory arrest. Resuscitative measures were unsuccessful.

Post Mortem Findings

The enlarged liver, spleen, kidneys, and lymph nodes were grossly infiltrated. Severe thymic atrophy was noted. Cutaneous and visceral pallor was present with cutaneous epicardial, renal, and peripelvic petechial hemorrhages. Right ventricular dilation and congestive pulmonary edema were observed.

Histology of the bone marrow revealed abundant cellularity with numerous blasts which lacked cytoplasm and often contained prominent nucleoli. Transition from primitive undifferentiated blast cells to numerous immature granulocytes was present, but maturation to mature polymorphonuclear leukocytes was lacking. These findings indicated acute myeloblastic leukemia of the null cell type.

Studies on Newly Diagnosed Leukemic Subjects

Blood samples were taken from nine newly diagnosed leukemic subjects at the time of admission to hospital for assay of triglyceride, cholesterol, ApoA-I, ApoB, ApoC-III, and ApoE levels. In addition, 10 additional newly diagnosed leukemic subjects had blood samples studied for triglyceride, cholesterol, and triglyceride fatty acid esters.

Methods

Quantification of apolipoproteins was performed by electroimmunoassay according to established procedures. The hyperlipemic sample was processed prior to assay utilizing a procedure to delipidize lipoproteins partially with butanol and di-iso-propyl ether. Cholesterol and triglyceride were analyzed by the Auto Analyzer II according to outlined procedures. Precipitation using heparin and manganese chloride was performed according to Burstin to separate HDL cholesterol. One ml serum samples from newly diagnosed leukemic children were lyophilized and dissolved in chloroform and methanol (2:1). The chloroform extract was evaporated to 50 μl and separated by thin layer chromatography using silica plates with a triglyceride standard. To separate the fatty acids, the samples from the silica plates in hexane were evaporated to dryness. Precisely 4.7 ml of 95 percent alcohol and 0.3 ml of 33 percent potassium
hydroxide were added, and the mixture was incubated at 60° for 30 minutes. Exactly 5.0 ml of water and 0.5 ml of 5.7 N hydrochloric acid were added, and the fatty acids were extracted twice with 2 ml of hexane. Quantification of the free fatty acids was determined by gas-liquid chromatography after methylation with boron trifluoride in methanol (Supelco) for 10 minutes at 100°C. The methylated fatty acids were extracted with n-hexane and injected into a Packard-Becker Model 421 gas chromatograph.25

Immunoglobins were assayed by nephelometry after reacting sera with monoclonal antibodies to IgG, IgA and IgM. Isolation of IgG was performed by affinity chromatography using Protein A coupled to sepharose.

Thrombin Clotting Time

A modified procedure based on the method established by Penner27 was used. One hundred μl of bovine thrombin (3.3 μ per ml) were added to 100 μl of patient plasma and diluted with 200 μl of 0.02 M Tris buffer (pH = 4) containing 0.15 M sodium chloride and bovine albumin (1 mg per ml).

Test for Lipoprotein Lipase Inhibition

Post-heparin plasma from a normal subject was obtained to separate lipoprotein lipase using heparin sepharose affinity chromatography.33 The lipolytic activity of the enzyme was measured with (14C) triolein emulsified in Triton X-100,33 and the patient's plasma was added to test for inhibition.

Very Low Density Lipoprotein Degradation

The VLDL substrate from the patient was tested for its capacity to be hydrolyzed in vitro by a known quantity of extracted human milk lipoprotein lipase.31 The reaction mixture consists of VLDL triglyceride (1 mg per ml), human milk lipoprotein lipase (eluted from the heparin-sepharose 4B column) (1 ml), bovine serum albumin (60 mg per ml), and 50 mM ammonium hydroxide-hydrochloric acid buffer (pH = 7.8, temp = 37°). Sampling for triglyceride was at 0, 2.5, 5, 7.5, 10, 15, 30, 40, 50, and 60 minutes. The degradation rate which follows first order kinetics was expressed as the half-time from which the K1 is calculated.

Results

The sample was noted to be grossly lipemic (triglyceride 782 mg per dl, cholesterol 175 mg per dl), and the lipid distribution in the major density classes indicated that the triglyceride was predominantly in the VLDL (87.3 percent). The VLDL:triglyceride ratio was 0.12, indicating that the VLDL was not relatively rich in cholesterol as in Type III hyperlipidemia.

Double diffusion analysis (in 1 percent agarose against antibodies to apolipoproteins A-I, A-II, B, C-I, C-III, D, E, and F) revealed that all the major Apo's were present, but that the A-I precipitin line was fainter than usual. When anti-IgG, anti-IgM, and anti-IgA antibodies were placed in adjacent wells, complete non-identity occurred between precipitin lines formed against apolipoproteins and the immunoglobulins. Quantification of apolipoproteins by electroimmunoassay revealed high levels of ApoC-III and ApoE, but deficiencies of ApoA-I and ApoB. ApoA-I was 41.8 mg per dl (106.0 ± 11.1); ApoB, 71.3 mg per dl (78.3 ± 13.9); ApoC-III, 10.0 mg per dl (5.8 ± 1.6); ApoC-II, 6.0 mg per dl (4.0 ± 1.0); ApoC-I, 6.9 mg per dl (7.0 ± 2.0); ApoE, 15.4 mg per dl (8.8 ± 2.0). Normal values and the standard error of the mean for one to three year olds are stated in parentheses, except for ApoC-I and
ApoC-II, where only adult normal values are available.

The ratio of ApoC-III in the heparin supernate (equivalent to HDL) to ApoC-III in the heparin precipitate (equivalent to LDL and VLDL) was 0.24 (normal range = 0.8 to 1.0), indicating a relative deficiency of ApoC-III in HDL, characteristic of the hypertriglyceridemia observed in Type V. While LpX was negative, Lp(a) was found to be present by double diffusion analysis.

**Anti-heparin Antibody**

When the leukemic patient’s IgG was isolated from a protein A affinity column and tested for reactivity to antibodies against whole serum, a single arc of precipitation was observed. The isolated IgG failed to reduce the thrombin clotting time. Pre-incubation of the sample with the patient’s IgG had no effect on the clotting time.

**Lipoprotein Lipase Inhibition**

The sample failed to significantly inhibit in vitro activity of both post-heparin plasma and milk lipoprotein lipase. Inhibition of lipoprotein lipase obtained from post-heparin plasma was 0 percent, whereas 7 percent inhibition of milk lipoprotein lipase was observed (not significantly different from the control).

**Very Low Density Lipoprotein Degradation by Milk Lipoprotein Lipase**

When the sample of isolated VLDL was tested for its rate of degradation by milk lipoprotein lipase, the rate was noted to be slower than for normal VLDL, but better than a VLDL sample from a Type V hyperlipidemia (figure 1). The first order kinetics ($K_t$) was obtained from the slope when log Co/Ct (increments in triglyceride degradation divided by time) was plotted against time. Leukemic patient $= 9.2 \times 10^{-3}$ min$^{-1}$

Type V hyperlipidemic patient $= 4.0 \times 10^{-3}$ min$^{-1}$

Normal $= 21 \times 10^{-3}$ min$^{-1}$

**Triglyceride Fatty Acids**

The percentage of 18:0 (stearic acid) in whole serum (table I) and VLDL (table II) triglyceride was increased and found to be more than double that in normal adults (pool) and in whole serum obtained from eight children of approximately two years of age. The data for normal VLDL triglyceride was similar to that of Wang et al$^{35}$ and Bierman et al$^7$ for normal and hyperlipidemic VLDL, respectively, with the exception of a tendency towards a higher percentage of saturated fatty acids in our normal subjects. When triglyceride fatty acids from eight subjects with newly diagnosed leukemia (ALL) were analyzed, they did not differ from normal.

**Immunoglobulins**

The IgG was 678 mg per dl (normal 424 to 1051); IgM, 66.4 mg per dl
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(normal 48 to 168); and IgA, 116 mg per dl (normal 14 to 123). No significant changes were observed following partial delipidization by the Cham-Knowles procedure, except for a slight increase in IgG to 701 mg per dl. The IgG isolated by a protein A affinity column reacted against antibody to whole serum on immunoelectrophoresis with one major arc of precipitation. Also, since LpX could not be detected, no other lipoproteins would have comigrated in a negative direction.

Subjects with Newly Diagnosed Acute Lymphoblastic Leukemia

The nine leukemic subjects were moderately hypertriglyceridemic with elevated ApoB in six cases and decreased ApoA-I in all cases (table III). One case had Down’s syndrome, which is also associated with mild hypertriglyceridemia, and one case had acute promyelocytic leukemia in contrast to the others with ALL. Six samples were taken at random times throughout the day and, therefore, were not fasting (table IV). Case 9 had acute promyelocytic leukemia and case 6 was associated with Down’s syndrome. When the eight additional cases were studied to determine whether or not their triglyceride composition was abnormal (table V), normal proportions of 14:0, 16:0, 16:1, 18:0, and 18:1 were observed.

Discussion

Extreme hyperlipidemia in association with leukemia has seldom been reported in man, with the exception of an acute blastic crisis in a 13 year old girl with chylothorax. Furthermore, the association of hyperlipidemia with other cancers is known to occur, but the mechanism has not been adequately defined. Therefore, this observation induced us to test sequentially whether antibodies to apolipoproteins, defects in endogenous heparin, inhibition of lipoprotein lipase, or delayed degradation of the patients’ VLDL were possible causes of the hyperlipidemia.

Delayed degradation of the patients’ VLDL suggested that it is a poor substrate for lipoprotein lipase, since fatty acid chain length and degree of unsaturation of the triglyceride fatty acid esters to a large extent influence LPL activity. The high proportion of stearic acid (18:0) in the patient’s VLDL would suggest that abnormal triglyceride composition resulted in its slower degradation. Alternatively, inhibition of enzyme activity is suggested by the relatively high ApoE level, but inhibitory activity could not be detected when the patient’s sample was tested for in vitro inhibition by an established method.

Because hypo- and hyperlipidemia are associated with myeloma and lupus, the possibility of an autoimmune mechanism was entertained. The presence of antibodies may possibly bind and mask the sites of enzyme attack when they are directed to apolipoproteins or possibly to lipoprotein lipase. In this study, no association could be detected between the apolipoproteins and immunoglobulins on double diffusion analysis. Also, after partial delipidization, there was no significant change in immunoglobulin levels, indicating that the delipidization procedure did not disassociate lipoprotein from immunoglobulin.

Cases with autoimmune hyperlipidemia have been studied by Beaumont, who demonstrated that soluble complexes agglutinated both HDL and LDL coated red blood cells after flotation by ultracentrifugation. This is indeed convincing evidence that antibodies precipitate with lipoproteins, but the normal binding of immunoglobulins to lipoproteins should be considered when interpreting these findings.

Since Glueck et al. demonstrated re-
sistance to the prolongation of the thrombin time by heparin in a case with hyperlipidemia and systemic lupus erythematosus, Beaumont et al.\textsuperscript{6} searched for a similar abnormality in hamsters with a nonmetastatic lymphoblastic tumor. Isolated immunoglobulin fractions from the hamster serum were tested for prolongation of the prothrombin time, which was corrected by the addition of heparin.\textsuperscript{6} In the present case, isolated IgM from the patient's serum failed to prolong the thrombin time, using a sensitive method, and ruling out the possibility of precipitation with endogenous heparin. Thus, our study appears to rule out the presence of antibodies to endogenous heparin, a factor which theoretically has a role in the release of lipoprotein lipase.\textsuperscript{19}

In addition, moderate hyperlipidemia has been observed in nine subjects with ALL, which is in agreement with Spiegel et al.\textsuperscript{32} This suggests that hypertriglyceridemia and a characteristic apolipoprotein profile (with elevated ApoB and ApoA-I levels) manifest in the early stages of the disease. However, the fatty acid composition of triglyceride from eight other newly diagnosed cases appeared normal, indicating that the triglyceride abnormality manifests at a later stage, as in our case.

These subtle lipoprotein abnormalities could be secondary to dietary lipid intake, defective hepatic and intestinal lipoprotein production, or defective disposal of triglyceride-rich lipoproteins. In addition, the disease process could involve the liver to such an extent that inhibition of remnant lipoprotein uptake occurs. This could contribute to the high ApoE level in the case studied, which is known to be associated with remnant removal disease.\textsuperscript{17} The relationship of these changes in lipid metabolism to cellular processes in tumor growth could be explored further utilizing animal models.\textsuperscript{4} Furthermore, since lipid metabolism has a role in cell-mediated and humoral immune attack on tumor cells,\textsuperscript{30} and since lipoproteins can regulate cholesterol biosynthesis by lymphocytes,\textsuperscript{11} it is possible that the observed aberrations in lipoprotein composition have a regulatory role in the neoplasia.

In summary, it has been shown by the present authors that hyperlipidemia in a case with advanced acute lymphoblastic leukemia was predominantly a hypertriglyceridemia, and this tendency appears to manifest in newly diagnosed cases of ALL. The possibilities have been excluded of antibodies to apolipoproteins, antibodies to endogenous heparin, and inhibition of lipoprotein lipase as possible causes. Delayed degradation of the patient's VLDL by milk lipoprotein lipase indicated that the triglyceride was a poor substrate for the enzyme. This was further substantiated by finding excessive saturated fatty acids (C 18:0) previously shown to result in a higher K\textsubscript{1} for the enzyme.

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