Characterization of Hypertriglyceridemia Induced by L-asparaginase Therapy for Acute Lymphoblastic Leukemia and Malignant Lymphoma*

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ABSTRACT

Plasma lipids and apolipoproteins were determined in 19 children with acute lymphoblastic leukemia (ALL) or malignant lymphoma (ML) who were treated by L-asparaginase with prednisolone and vincristine. Extreme hypertriglyceridemia, i.e., over 10,000 mg/l of the maximum serum triglyceride concentration, was induced in 8 patients; these concentrations were not over 10,000 mg/l in the remaining 11 patients. The possibility was raised that the apolipoprotein E (apoE) isoform apoE4 (ε4) participated in the induction of extreme hypertriglyceridemia, since the frequency of the apoE4/E3 phenotype in the patients with extreme hypertriglyceridemia was higher compared to those in the patients without extreme hypertriglyceridemia and control subjects (n = 248). The acute and severe hypertriglyceridemia was induced at 8 to 14 days after the end of the L-asparaginase therapy, with an earlier remarkable increase in the apoCIII/apoCII ratio and an extreme decrease of fibrinogen concentrations (a marker of the protein productivity of the liver). It is well known that apoCII and apoCIII have possible functions as an activator and an inhibitor of lipoprotein lipase (LPL), respectively. The extreme increase in the apoCIII/apoCII ratio could be one of the reasons for the accumulation of triglyceride-rich lipoproteins in plasma.

Introduction

L-asparaginase is used for remission induction chemotherapy in children with acute lym-
phoblastic leukemia (ALL) or malignant lymphoma (ML). It catalyzes the hydration of L-asparagine to L-aspartic acid and ammonia, and, consequently, suppresses the proliferation of tumors such as ALL, which require L-asparagine as a nutrition substance and are defective in the synthetase of L-asparagine.\textsuperscript{1} L-asparaginase is known to inhibit the synthesis of many proteins in the liver.\textsuperscript{1,2} There are several reports of changes in hemostatic and fibrinolytic proteins caused by treatment with L-asparaginase.\textsuperscript{3,4,5,6} It is commonly used in combination with other drugs such as vincristine, metotrexate, and prednisolone.

It was also recently demonstrated that a remarkable hyperlipidemia of Type IV or Type V was induced as a side effect of this L-asparaginase therapy.\textsuperscript{7,8} It was suggested by monotherapy results that the hyperlipidemia was caused by L-asparaginase itself. This side effect may be explained by a reduced synthesis of lipolytic enzymes (such as hepatic triglyceride lipase), which determine plasma triglyceride levels.\textsuperscript{8} An alternative possibility is interference with the synthesis of apoE receptors in the liver followed by an accumulation of chylomicron remnants. However, it is well known that prednisolone also induces hypertriglyceridemia by accelerating lipoprotein synthesis, and that serum triglyceride concentrations positively correlate with the cumulative prednisolone dose.\textsuperscript{9} The pathogenetic mechanism of extreme hypertriglyceridemia induced by L-asparaginase therapy, however, remains to be determined.

In the present study, the hypertriglyceridemia induced by L-asparaginase was characterized in order to clarify its possible mechanism.

**Patients and Methods**

**Patients**

Nineteen patients, 14 diagnosed with ALL and 5 diagnosed with ML, aged 1 to 18 years old (mean 9.8 years), received induction chemotherapy in which prednisolone and vincristine were given together with L-asparaginase (derived from *Escherichia coli*). Each of these patients was treated by one of several protocols, which basically consisted of 3 repeats of L-asparaginase treatment for 3 or 4 consecutive days with a one-week interval. Blood samples were obtained for the determination of serum lipids and apolipoproteins before and after the start of chemotherapy at 2-day to one-week intervals.

**Assay of Lipoprotein Profile**

Lipoprotein profiles were determined by agarose gel electrophoresis using a commercially available kit.\textsuperscript{*} Briefly, 0.5 to 1.0\mu L of serum was applied on a 1 percent agarose gel film using a sample template. After electrophoresis, separated lipoproteins were fixed by the drying of the gels and were stained by Fat Red 7B.

**Analytical Procedures**

Serum cholesterol and triglycerides were measured by enzymatic methods using a commercially available test kit.\textsuperscript{†} Apolipoproteins were determined by turbidimetric immunoassay kits.\textsuperscript{‡} Kinetic techniques based on the thrombin time were used for the quantitative assay of plasma fibrinogen.\textsuperscript{10}

**Assay of apoE Phenotype**

ApoE phenotypes were determined by a combination of isoelectric focusing (IEF) and immunoblotting as described previously.\textsuperscript{11} Serum samples were incubated with neuraminidase followed by treatment with \beta-mercaptoethanol to obtain clear IEF patterns. The “Fisher exact test” was applied to compare the apoE phenotype frequencies among the groups.

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Results

The frequencies of apoE phenotypes were determined in the patients in whom extreme hypertriglyceridemia was (n = 8) or was not (n = 11) induced by L-asparaginase therapy. The higher frequency (50.0 percent) of apoE phenotype E4/E3 in the patients with extreme hypertriglyceridemia was observed as compared with the frequency in the patients without hypertriglyceridemia (18.2 percent) and in the control subjects (20.6 percent) reported previously. However, a statistically significant difference was not observed. In addition, no significant relationship was indicated between the triglyceride levels and the four kinds of apoE phenotypes associated with extreme hypertriglyceridemia induced by L-asparaginase therapy (data not shown).

The change of lipoprotein profiles in the patients with hypertriglyceridemia was determined by agarose gel electrophoresis (figure 1). Chylomicron and chylomicron remnant were observed in parallel with a remarkable increase of triglycerides.

The profiles of total cholesterol levels and apoCII, apoCIII, and apoE, which are the main apolipoproteins of triglyceride-rich lipoproteins, were associated with that of triglyceride, as shown for 3 of the patients with extreme hypertriglyceridemia in figure 2 and figure 3, respectively. Although the total cholesterol levels had some resemblance to the triglyceride levels, the former decreased at a leisurely rate compared with the latter after the maximum triglyceride levels were reached. The peaks of apoE and apoCIII were simultaneous with or just behind the peak of triglyceride; the appearance of the apoCII peaks was more delayed. The monotherapy of prednisolone also induced a slight hypertriglyceridemia; however, the apoCIII/apoCII ratio was changed in parallel with that of triglyceride (figure 4).

In figure 5 are shown the profiles of serum triglyceride, fibrinogen concentrations, and apoCIII/apoCII ratio in 3 patients with and 3 patients without extreme hypertriglyceridemia. The extreme hypertriglyceridemia was induced at nearly 10 days after the end of the L-asparaginase therapy. The fluctuations of the fibrinogen concentration were in sharp contrast to those of the apoCIII/apoCII ratio, especially in the patients with extreme hypertriglyceridemia. The two fluctuation curves crossed each other just after the maximum triglyceride levels were reached.

Discussion

A previous report suggested that the reduction of lipoprotein lipase (LPL) or apoE recep-

<table>
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</tr>
<tr>
<td>3280</td>
<td>12440</td>
</tr>
<tr>
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![Figure 1](image-url) Changes of lipoprotein profiles caused by the extreme increase of triglyceride after L-asparaginase therapy.
tor synthesis could induce hypertriglyceridemia as a side effect of L-asparaginase therapy. It is well known that the catabolism of remnant particles such as the chylomicron remnant is mediated by apoE receptors. If the hypertriglyceridemia was caused by the reduction of apoE receptor synthesis in the liver, a remarkable accumulation of chylomicron remnants as shown in type III hyperlipoproteinemia would be observed after treatment with L-asparaginase. However, the present lipoprotein profiles on agarose gel and the cholesterol/triglyceride ratios revealed that chylomicrons were accumulated in plasma at the early stage of hyperlipidemia. This indicates that L-asparaginase inhibited the hydrolysis of the chylomicrons.

However, the possibility that the apoE receptor synthesis was partially inhibited followed by an effect on the triglycerides level cannot be denied, since the fibrinogen synthesis (as a marker for productivity of the liver) was suppressed by L-asparaginase, and its concentration reached its nadir prior to the extraordinary increase of triglycerides. In one patient in whom hypertriglyceridemia was
induced, a normal level of lipoprotein lipase (LPL) activity was determined at the point when the triglycerides were markedly increased (data not shown). These results suggest that the suppression of LPL or apoE receptor synthesis is not an essential trigger of the hypertriglyceridemia.

The present results prompted the hypothesis that the hypertriglyceridemia is induced by a suppression of LPL activity as a result of an increase in the apoCIII/apoCII ratio, since apoCII is an important co-factor for LPL and an excess amount of apoCIII inhibits its activity. It was particularly interesting that the severe hypertriglyceridemia was always observed, if developed, at nearly 10 days after the end of the third administration of L-asparaginase therapy.

In case 2, the decrease of apoCII was one of the interesting responses in the early stage of the accumulation of triglycerides, whereas apoCIII increased in parallel with the triglyceride levels. After apoCIII increased, apoCII increased, followed by the decrease in triglycerides. These changes were sensitively indicated by the variation of the apoCIII/apoCII ratio. The similar tendency was observed even when the triglycerides increased only a small amount during the L-asparaginase therapy. The cause of these differences between the two groups (extreme hypertriglyceridemia induced and not induced) remains to be determined. The profile of the apoCIII/apoCII ratio in the mild hypertriglyceridemia induced by the prednisolone monotherapy was quite different from that induced by the L-asparaginase therapy. The synergistic effect of L-asparaginase and prednisolone could cause, in part, extreme hypertriglyceridemia.

No relationship was observed between the absolute values of the apoCIII/apoCII ratio and the triglyceride levels. This was clarified by case 3, whose maximum apoCIII/apoCII ratio was 4.0 among the measured values. Higher apoCIII/apoCII ratios were sometimes observed in the patients without severe hypertriglyceridemias; however, the maximum ratios in those cases were usually observed in the midst of the 3 administrations of the L-asparaginase treatment, when the synthesis of all proteins in the liver could be suppressed.

It is well known that apoE is associated with triglyceride-rich lipoprotein catabolism and exists as three major isoforms (E4, E3, and E2) which represent three homozygous (E4/E4, E3/E3, and E2/E2) and three hetero-

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**Figure 4.** Time courses of changes in serum triglyceride (●) and apoCIII/apoCII ratio (○) in a patient treated with prednisolone monotherapy. M-Pred, Methylprednisolone. Pred, Prednisolone.
zygous (E4/E3, E4/E2, and E3/E2) phenotypes. In this study, the possibility was raised that the apoE4 (e4) isoform participated in the induction of extreme hypertriglyceridemia. However, no clear relationship was confirmed between the apoE phenotype and the extent of hypertriglyceridemia, because of the small number of patients investigated.

**References**

1. Capizzi RL, Bertino JR, Skeel RT, Creasey WA, Zanes R, Olayon C, Peterson RG, Handschumacher RE.


4. Priest JR, Ramsay NK, Bennett AJ, Krivit W, Edson JB. The effect of L-asparaginase on antithrombin, plasminogen, and plasma coagulation during therapy