A new enzyme-linked immunosorbent assay system for human hepatic triglyceride lipase

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ABSTRACT

Background: The objective of this study was to establish a new sandwich based enzyme linked immunosorbent assay (ELISA) for measuring the protein mass of human hepatic triacylglyceride lipase (HTGL).
Method: Two mouse monoclonal antibodies raised against human HTGL were used for the sandwich ELISA. The post-heparin plasma (PHP) samples obtained at a heparin dose of 50 unit/kg from 124 normolipidemic subjects were used for this ELISA.
Results: The dynamic assay range of the developed ELISA for the HTGL was from 0.47 to 30 ng/ml. The CV was <7% in both intra- and inter-assays, and it did not cross-react with lipoprotein lipase or endothelial lipase (EL). The HTGL concentration in PHP showed a strong correlation with HTGL activity [n = 121, r = 0.778, p < 0.001]. There was a weak relation of HTGL concentration against high-density lipoprotein cholesterol (HDL-C) [n = 123, r = −0.229, p = 0.011] but no relations against total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), small dense LDL, remnant like particles cholesterol (RLP-C) and RLP-TG were confirmed. Interestingly, a weak but positive correlation between HTGL concentration and EL concentration was shown [n = 122, p = 0.013, r = 0.224].
Conclusion: These results indicate that this new sandwich ELISA for measuring HTGL concentration in PHP can be applied in a daily clinical practice.

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1. Introduction

Hepatic triglyceride lipase (HTGL), a lipolytic enzyme, is a secreted glycoprotein, and is synthesized by hepatocytes and bound to heparin sulfate proteoglycans at the surface of liver sinusoidal capillaries. HTGL plays a major role in lipoprotein metabolism as a lipolytic enzyme that hydrolyzes triglycerides (TGs), phospholipids in chylomicron remnants, intermediate-density lipoproteins (IDLs) and high-density lipoproteins (HDLs) [1,2]. Patients with HTGL deficiency present with hypercholesterolemia or hypertriglyceridemia, and accumulate very-low-density lipoproteins (VLDLs), chylomicron remnants, IDLs, TG-rich low-density lipoproteins (LDLs) and HDLs [3–8].

Although there was a commercially available sandwich ELISA kit that was developed in Japan [9] for measuring HTGL concentration assay system using 2 distinct monoclonal antibodies raised against human HTGL purified from post-heparin plasma (PHP) as a starting material, it was withdrawn from the market with unknown reasons almost a decade ago. Since no high sensitive assay system for measuring the protein concentration in plasma was available, we reported on an easy and quick PHP–HTGL activity assay system several years ago [10,11]. But we believe that it is still important to determine quantity of HTGL concentration in order to understand the clinical significance of this enzyme in lipoprotein metabolism, especially in the case of detecting dysfunctional types of HTGL. We have now established a new sandwich ELISA method using 2 distinct monoclonal antibodies which are raised against purified human HTGL from a concentrated conditioned medium of human HTGL-471 transfected Chinese hamster ovary (CHO) cells.

BSA

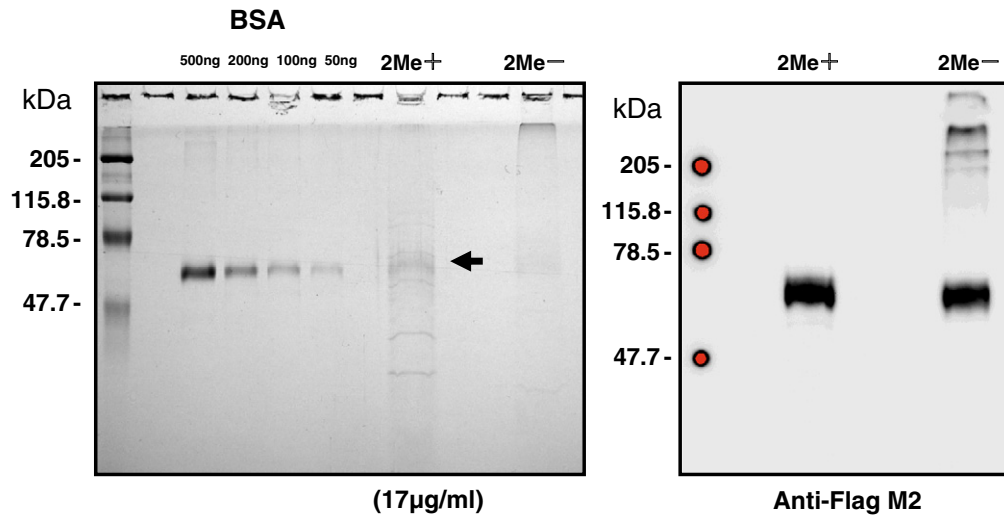(17µg/ml)   Anti-Flag M2

Fig. 1. Quantification of purified human HTGL protein and immunoblotting analysis. We quantified the purified Flag tagged HTGL-471 protein from the concentrated conditioned medium of human HTGL-471/CHO 3B1 using an anti-FLAG M2 affinity gel and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE, right side) followed by staining with Coomassie Brilliant Blue (left side). The purity of the recombinant human HTGL protein was determined at the concentration of 17 µg/ml by densitometry using a Multi gauge and the concentration of the protein was determined by comparison with BSA as an indicator.

2. Materials and methods

2.1. Preparation of purified HTGL

The purified human HTGL was prepared as previously reported [12]. We generated recombinant human HTGL as follows. A full-length of human HTGL cDNA was identified in human liver cDNA (Clonetech) by PCR using primers in which a FLAG-epitope tag was added to the 3′ end of cDNA before sub-cloning. To generate the plasmid encoding hHTGL-Full, the cDNA was inserted into pcDNA3.1(+) expression vector (Invitrogen). However, human HTGL is exhibited on cell surface through the binding of 5′ carboxyl-terminal residues (KRKIR) [12]. Therefore, we also prepared a truncated human HTGL mutant (hHTGL-471) this time by using deleted 5′ carboxyl terminal residues in order to promote its secretion in culture supernatant. Then, we used antisense PCR primers for generating the plasmids encoding hHTGL-Full and hHTGL-471, respectively. Each PCR product was inserted into pcDNA3.1(+) expression vector after a FLAG-epitope tag was added to the 3′ end. CHO cells were transfected with the hHTGL-Full plasmid or the hHTGL-471 plasmid and selected with 500 µg/ml of G418 for establishing of stable transfectants. Next, we screened the transfectants by detecting the expression of hHTGL-Full or hHTGL-471 expression with anti- c-FLAG rabbit IgG (Immuno-Biological Laboratories) and selected the high-expression clones as a human HTGL-Full-Flag/CHO named as 8A4 or human HTGL-471-Flag/CHO named as 3B1, respectively. We purified HTGL protein from the concentrated conditioned medium of human HTGL-471-Flag/CHO 3B1 using an anti-FLAG M2 affinity gel (Sigma-Aldrich). We estimated the purity of the recombinant human HTGL protein using the Multi Gauge

densitometry and determined the concentration of the protein by comparison with bovine serum albumin (BSA) as an indicator after electrophoresis (Fig. 1).

2.2. Preparation of monoclonal antibody (MoAb)

The monoclonal antibody for human HTGL was prepared as reported previously [10]. In brief, we used the purified HTGL protein which was emulsified with Freund complete adjuvant for first immunization and then immunized it with Freund incomplete adjuvant after 2 weeks. It was immunized into BALB/c mice (Charles River) 2 times with Freund incomplete adjuvant every week. After the immunizations, we removed the spleen from the mice and hybridized it with mouse myeloma cells X63-Ag 8.653 as a fusion partner. We generated monoclonal antibodies up to 36 clones, and finally selected the best 2 monoclonal antibodies named 26A1 MoAb and 31A1 MoAb

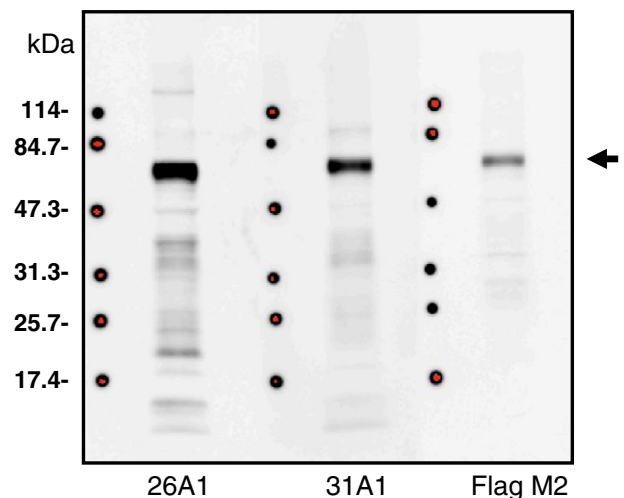26A1   31A1   Flag M2

Fig. 2. Demonstration of the specificities of anti-human HTGL mouse monoclonal IgGs by western blotting. For immunoblotting, 5 µl conditioned medium of human HTGL Full-Flag/CHO 8A4 was used for 12.5% SDS–PAGE and transferred to nitrocellulose membrane (Bio-Rad). The membrane was incubated with 2 µg of 26A1 MoAb or 31A1 MoAb followed by incubation with secondary antibody conjugated with horse-radish peroxidase.

Table 1
Clinical profile of the study subjects.

Age, y	26.4 ± 6.1
Body mass index, kg/m^2	25.0 ± 3.7
Men/women	67/57
Total cholesterol, mg/dl	163 ± 30
Triglycerides, mg/dl	57.2 ± 37.3
HDL-cholesterol, mg/dl	48.5 ± 11.8
LDL-cholesterol, mg/dl	93.3 ± 26.4
sdLDL, mg/dl	26.7 ± 12.7
RLP-cholesterol, mg/dl	4.1 ± 2.1
RLP-triglycerides, mg/dl	7.1 ± 10.6