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Identification of Insulin-like Growth Factor (IGF)-I and IGF-Binding Protein in Chylous Ascites

Insulin-like growth factors (IGFs) are bound by several IGF-binding proteins (IGFBPs) that appear to regulate IGF transportation, receptor binding and action. In adult human serum, most of IGFs are bound in a 150 kDa complex which could not cross the capillary wall. We measured IGF-I and IGFBPs in chyle by radioimmunoassay and western ligand blot. The concentration of IGF-I in chyle was only 15% of the corresponding serum level and most of IGF-I was found in 50 kDa complex. The IGFBPs profile in chyle, especially IGFBP-3, was different from that of serum. The concentration of IGFBP-3 in chyle was much less than in serum and the size of glycosylated IGFBP-3 was different from that of serum. However, the size and relative amount of IGFBP-1 and -2 in chyle were similar to serum. This finding indicates that IGF-I and IGFBPs in chyle to a large extent originate in the vascular system and only the 50 kDa complex can cross the capillary barrier.

Key Words : *Insulin-like growth factor-I, IGF binding proteins, Chyle*

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INTRODUCTION

Insulin-like growth factors, IGF-I and -II, play essential roles in the modulation of cellular growth and the differentiation of various organs (1, 2). Each peptide interacts with a distinct high affinity for a family of IGF-binding proteins (IGFBPs). To date, six different IGFBPs (IGFBP-1 to -6) have been identified, cloned and sequenced, and these are believed to modulate the effects of IGFs at target tissues in an autocrine/paracrine system (3-6). In human adult serum, most IGF is bound in a 150 kDa complex composed of IGF-I or IGF-II, acid-labile subunit (ALS) and IGFBP-3 (7, 8). IGFs are also found in most biological fluids tightly bound to several IGFBPs.

The goal of this study was to identify IGF-I and IGFBPs in chyle, and confirm the notion of a capillary barrier to IGFs by comparing IGF-I and IGFBPs distribution in the blood with that in chyle.

MATERIALS AND METHODS

Sample

Chylous ascites was collected from an 3 month old infant with massive chylous ascites secondary due to congenital leaky lymphatics. The ascites showed milky appearance and the result of biochemical studies was the same as those in chyle and lymph. The chyle consists of lymph and droplets of triglyceride fat in a stable emulsion, and the protein concentration of chyle was 2.8 times higher than that of serum (13 vs 4.6 g/dl). The serum was also taken simultaneously and control sera were collected from 10 healthy adults.

Reagents

Recombinant human IGF-I was purchased from Bachem (Torance, CA, USA). IGF-I was iodinated by a modification of the chloramine T method to a specific activity of 150-300 μCi/μg. Polyclonal anti-IGF-I anti- serum, a generous gift of Drs. LE Underwood and JJ Van Wyk (University of North Carolina at Chapel Hill) was distributed through the Hormone Distribution Program of NIDDK to National Hormone and Pituitary Program. Polyclonal anti-sera to the *E. coli*-derived, non-glycosylated IGFBP-3 was kindly provided by Dr. Rosenfeld (Oregon Health Sciences University, Oregon, USA). Alkaline phosphatase conjugated goat anti-rabbit sera, nitro blue tetrazolium (NBT) and bromochloroindolyl phosphate (BCIP) were purchased from Sigma (St. Louis, MO,

USA).

IGF-I Radioimmunoassay (RIA)

To separate IGF peptides from their binding proteins, 500 μl of sample was chromatographed in 0.1% formic acid on 1.0×100 cm column containing Sephadex G-50 fine. The fractions eluting between 50 and 70 ml, which containing 90% of the IGF peptide activity, were collected in a glass tube containing 1.0 ml of 1% BSA, lyophilized and reconstituted 1 ml in RIA buffer. Chyle and serum IGF-I concentrations were determined by RIA using ^{125}I-IGF-I and polyclonal anti-somatomedin-C antiserum.

Western ligand blot (WLB)

Four μl of serum or 4 and 20 μl of chyle were electrophoresed on 10% SDS-PAGE under non-reducing condition with a molecular weight marker (Biorad, Richmond, CA, USA). Electrophoresed proteins were electroblotted onto nitrocellulose, incubated 2×10^6 cpm of ^{125}I-IGF-I and exposed to x-ray film for 7 days according to the method of Hossenlopp et al. (9).

Western Immunoblot (WIB)

Four μl of serum or 4, 10 and 20 μl of chyle were electrophoresed on 10% SDS-PAGE under non-reducing condition, electroblotted onto nitrocellulose and blocked with 3% non-fat milk solution. The nitrocellulose was incubated for 2 hours with anti-IGFBP-3 antibody and washed three times in 0.1% Tween 20 buffer. Following 1 hour incubation with goat anti-rabbit IgG conjugated with alkaline phosphatase, the nitrocellulose was washed in 0.1% Tween 20 buffer and stained with NBT and BCIP.

Neutral size-exclusion chromatography

To separate 150 and 50 kDa of IGF-IGFBP complexes, 500 μl of serum or chyle were applied to a Sephacryl S-200 column (1.0×100 cm) equilibrated in 0.05 M sodium phosphate buffer, pH 7.4, and calibrated with γ-globulin (158 kDa), hemoglobin (65 kDa), ovalbumin (44 kDa), cytochrome C (14 kDa) and iodinated IGF-I (7.5 kDa). The sample was eluted with 0.05 M sodium phosphate buffer at a flow rate of 15 ml/hour, and the fractions containing 150 and 50 kDa complexes were pooled separately, lyophilized and reconstituted 1 ml in distilled water. To measure the concentration of IGF-I in 150 and 50 kDa complexes, acid column chromatography and IGF-I RIA were performed as described above.

Table 1. IGF-I level in serum and chyle from an infant with chylous ascites

Samples	IGF-I (ng/ml)		
	Total	150 kDa (%)	50 kDa (%)
Serum	85.2	63.4 (74.4)	21.8 (25.6)
Chyle	12.7	1.9 (14.9)	10.8 (85.1)
Chyle / Serum	0.15	0.03	0.34
Pooled adult serum	306.3	246.8 (80.6)	59.5 (19.4)

RESULT

The concentration of IGF-I in serum and chyle, as determined by RIA was provided in Table 1. The serum IGF-I concentrations were 306.3 ng/ml in adult, and 85.2 ng/ml in infant. Although there was a low serum concentration of IGF-I in the infant, the proportion of IGF-I found in 150 kDa complex was similar to that of pooled adult serum (74 vs 81%). The concentration of IGF-I in chyle was 12.7 ng/ml, and it was only 15% of the corresponding serum level. Furthermore, most of IGF-I in chyle was found in 50 kDa complex.

A western ligand blot of chyle and serum from an infant with chylous ascites was provided in Fig. 1. Pooled adult serum (lane 1) had two major bands, corresponding to the glycosylated form of IGFBP-3, with molecular weight (MW) ranging between 37-43 kDa. Other bands were seen in control serum with MW of 31, 28 and 24 kDa, corresponding to IGFBP-2, -1 and -4, respectively. Serum from infant (lane 2) showed similar IGFBPs profile with pooled adult serum, but the concentration of

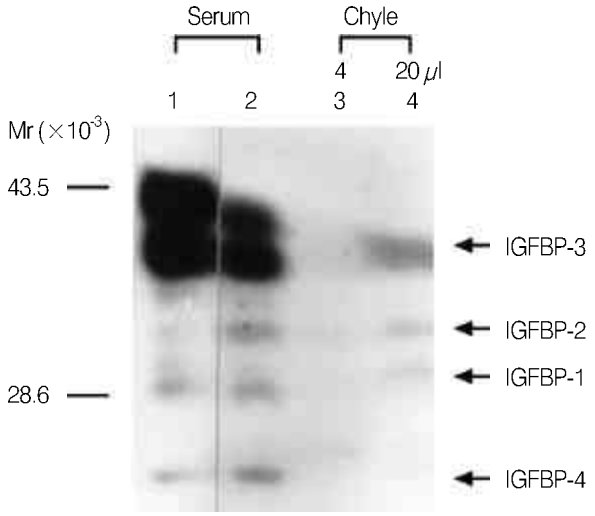

Fig. 1. Western ligand blot of serum and chyle. Lane 1, pooled adult serum; lane 2, serum from an infant with chylous ascites; lane 3 and 4; chyle from an infant with chylous ascites. Arrows indicate IGFBP-1, -2, 3 and -4.

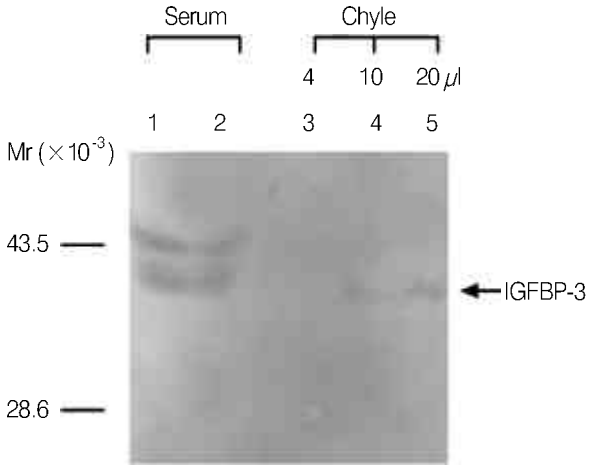

Fig. 2. Western immunoblot of serum and chyle. Lane 1, pooled adult serum; lane 2, serum from an infant with chylous ascites; lanes 3, 4 and 5; chyle from an infant with chylous ascites. Arrow indicates IGFBP-3.

IGFBP-3 was lower than that of adult serum. At identical volumes of serum and chyle, we could not detect any IGFBP band in chyle (lane 3). With an increased volume of chyle (20 vs 4 μl serum), comparison was easier. Using this amount of chyle, we found 4 IGFBP bands (lane 4). As you can see, the major band was 37-40 kDa sized single band and this band seemed to be IGFBP-3. To determine this band was IGFBP-3, we did WIB with anti-IGFBP-3 (Fig. 2). In serum (lanes 1 and 2), most of immunoreactive material was presented in 37-43 kDa band and this band was correlated with WLB. As expected, 37-40 kDa sized IGFBP band in chyle was IGFBP-3 (lanes 4 and 5). The relative portion of IGFBP-3 were lower in chyle than in serum, whereas those of IGFBP-2 and -1 bands were similar in chyle and serum. The concentration of IGFBP-4 in chyle was very low and it is hard to see 24 kDa sized band on WLB.

DISCUSSION

Insulin-like growth factor (IGF)-I and -II exert a variety of metabolic and anabolic effects on skeletal and soft tissues (1, 2). IGF-I is primarily produced in the liver under the control of GH and circulates in the plasma bound to one or more IGF-binding proteins (IGFBPs). IGFBPs bind IGF-I and -II with high affinity and specificity and are thought to play a major role in the modulation of the cellular binding and action of IGFs (3, 4, 10).

In the present study, the IGF-I and IGFBPs were identified in chyle. The concentration of IGF-I and IGFBP-3 in chyle is very low compared with serum. The concentration of IGF-I in chyle was only 15% of the corresponding serum level and it was similar to that of human milk (11, 12) or lymph (13, 14). Although IGFBPs which were found in serum existed in chyle, IGFBP-3 was relatively lower in chyle than in serum and than in other IGFBPs. The ratio of IGFBP-3 to IGFBP-2 was greatly reduced in chyle compared to serum. Most of the IGFs in adult serum exist in the 150 kDa complex, consisting of IGFs, IGFBP-3 and acid labile subunit (ALS), and smaller amounts of IGFs are bound to IGFBPs as complex of 50 kDa (7). In this study, most of IGF-I in serum was found to be eluted in the 150 kDa IGF-IGFBP complex. In contrast, only 15% of IGF-I in chyle was detected in the 150 kDa complex, and most of IGF-I was found in 50 kDa complex. These findings suggest that IGF-I and IGFBPs in chyle to a large extent originate in the vasculasr system. Furtheremore, it would seem that the large complex of serum does not cross the capillary barrier, and this finding consists with other reports (13, 15). Whereas most of IGFBP-3 in human serum exists as 37-43 sized glycosylated doublet, IGFBP-3 in chyle is existed as 37-40 sized single band. Therefore, some degradation of IGFBP-3 may take place in chylous ascites. However, we could not exclude the possibility of local production of IGFBP-3 which is glycosylated at different site from that of serum. The concentration of IGF-I in chyle was one half of that found in the 50 kDa complex of serum but IGFBP-1 and -2 levels measured by WLB were similar to that of serum. This finding suggests that there is some restriction to their passage over the capillary barrier.

Since the properties of chyle (IGF-I content, WLB profile of IGFBPs) appear to be a similar to those of the serum 50 kDa complex, it can be suggested that the IGFBPs and IGFs with which they form the monomeric complex around 50 kDa do cross the capillary barrier. The fact that the 150 kDa IGF-IGFBP complex cannot cross the capillary barrier, whereas the 50 kDa complex can, suggest that the function of the former would provide circulating reserves of IGFs, whereas the latter would serve to transport the IGFs to their target cells.

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