Serum Insulin-like Growth Factor Binding Protein-6 (IGFBP-6) Levels in Various Clinical Conditions

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Introduction

There are few reports on serum levels of insulin-like growth factor binding protein-6 (IGFBP-6) (1). Recently we have developed Western immunoblot of IGFBP-6. In the present study, to investigate the clinical significance and regulation of serum IGFBP-6, we measured serum IGFBP-6 in various clinical conditions by Western immunoblot.

Materials and Methods

Serum and Urine Samples

Serum samples were obtained from normal subjects, patients with growth hormone deficiency (GHD), acromegaly, and chronic renal failure (CRF). To investigate the effects of IGF-I on serum IGFBP-6, we measured serum IGFBP-6 in normal subjects who received sc administration of IGF-I (0.1 mg/kg) once a day for 7 consecutive days (2). Spot urine samples were collected from two normal adults; 20 mL urine samples were dialyzed against distilled water overnight and lyophilized. The samples were reconstituted with distilled water and then passed through

a desalting column (Ampure™ SA). The desalted samples were used for IGFBP-6 Western immunoblot.

Western Immunoblot for IGFBP-6

Serum or urine samples were electrophoresed on 12 % SDS-acrylamide gel under non reducing conditions. The size-fractionated proteins were electroblotted onto a nitrocellulose sheet. The sheet was incubated with anti-IGFBP-6 antibody (Austral Biologicals, CA), washed, and then incubated with horse raddish peroxidase-conjugated anti-rabbit IgG. IGFBP-6-anti-IGFBP-6 antibody complexes were detected with Enhanced Chemiluminescence (ECL) system.

Results

Serum IGFBP-6 Values

Immunoreactive band of IGFBP-6 in normal serum was recognized at 30kDa, and the band was displaced by unlabeled IGFBP-6. Therefore, specific IGFBP-6 in serum was detected in this system.

Effects of GH or IGF-I on Serum IGFBP-6

Using this system, IGFBP-6 in sera from patients with GHD, acromegaly, and from

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normal subjects who received IGF-I for 7 consecutive days were studied to investigate whether GH or IGF-I regulate serum IGFBP-6 levels or not.

When sera from patients with GHD were studied, serum IGFBP-6 did not change before and after GH treatment. Furthermore, serum IGFBP-6 levels did not change after successive adenomectomy in patients with acromegaly and after repetitive IGF-I administration for 7 days in normal adults.

IGFBP-6 Values in Various Clinical Conditions

Compared with the other clinical conditions studied, serum IGFBP-6 levels were remarkably elevated in patients with CRF.

Next we investigated whether increased levels of IGFBP-6 in patients with CRF change either after hemodialysis or renal transplantation. However, the levels did not change after hemodialysis. However, serum IGFBP-6 levels decreased one day after renal transplantation and decreased further with the improvement in renal function.

Urinary IGFBP-6

IGFBP-6 in urine from normal subjects was studied as we speculated that increased IGFBP-6 levels in CRF might be related to the retention of IGFBP-6 in serum. IGFBP-6 was detected in normal urine samples.

Discussion

In this study, IGFBP-6 values in serum and urine samples were measured using IGFBP-6 Western immunoblot.

- 1) Immunoreactive IGFBP-6 was detected at 30 kDa in serum and urine using this system.
- 2) Serum IGFBP-6 levels were not GH nor IGF-I dependent.
- 3) Serum IGFBP-6 levels remarkably increased in patients with chronic renal failure and the levels did not change after hemodialysis. However, the IGFBP-6 levels dramatically decreased already one day after renal transplantation, and decreased further with the improvement of renal function.

These data indicate that IGFBP-6 might be excreted by the kidneys and serum IGFBP-6 levels might be related to renal function.

References

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