Plasma Levels of Insulin-Like Growth Factor Binding Protein-3 in Acute Trauma Patients

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Insulin-like growth factors (IGFs) are a family of polypeptides that regulate cell growth. Their action and bioavailability are modified by binding proteins such as IGF binding protein-3 (IGFBP-3). Plasma IGFBP-3 level was found to be growth hormone (GH)-dependent, which makes detection of IGFBP-3 useful in the evaluation of GH secretion. In the early catabolic flow phase of severe injury, when plasma levels of GH and IGF-1 are low versus uninjured levels, the role of IGFBP-3 has not been investigated. We have measured basal levels of these polypeptide hormones in 16 adult (13 men and three women aged 47 ± 7 years) severely injured (Injury Severity Score, 32 ± 2), hypermetabolic resting energy expenditure [REE] to basal energy expenditure [BEE] ratio, 1.30 ± 0.05), ventilator-dependent, multiple-trauma patients within 48 to 60 hours of injury when the patients were receiving maintenance fluids without calories or nitrogen. These basal values were compared with those of 16 age-matched postabsorptive normals. In the catabolic flow phase of injury, plasma levels of GH, IGF-1, and IGFBP-3 were significantly reduced by 50%, 46%, and 45%, respectively. There was a significant linear inverse relationship between IGFBP-3 and age and also a positive correlation between IGFBP-3 and IGF-1 in both control and injured subjects. The ratio of IGFBP-3 to IGF-1 was not changed in trauma victims. Measurement of plasma IGFBP-3 levels has potential as a marker for monitoring GH therapeutic efficacy.

THE EXISTENCE OF circulatory binding proteins for peptides of the insulin-like growth factor (IGF) family has been recognized for almost two decades.[1,2] The ability to quantify these proteins is vital to a complete understanding of growth regulation by IGFs, since changes in their concentrations will affect the size distribution of the circulating growth factors and perhaps its availability to tissues. In addition, as it may directly or inversely reflect growth hormone (GH) status, measurement of these proteins could provide information of value in the diagnosis and monitoring of growth disorders. In humans, the majority of IGFs circulate bound to IGF binding proteins (IGFBPs), particularly IGFBP-3, and less than 1% is free.[3] This binding prolongs the metabolic half-life of IGFs in the circulation and serves as a metabolic reservoir of IGFs. Recent data support a model of the GH–IGF-1–IGFBP-3 axis that is closely regulated by the metabolic status of the individual, which thereby controls bioavailability of IGF-1.[4]

GH deficiency is typically characterized by low circulating levels of IGF-1. Treatment of GH deficiency by administration of GH usually results in an increase in IGF-1 levels and an improvement in growth rate.[5] Multiple studies have demonstrated that IGFBP-3 is GH-dependent[6,7] and that IGFBP-3 levels are not significantly affected by food intake and show little diurnal variation.[8] Age-associated inverse changes in plasma IGFBP-3 levels[9] parallel trends observed in plasma IGF-1 and GH levels in healthy women. IGFBP-3 plasma concentration is reduced in hypophysectomized animals and is increased by administration of GH.[10,11] It is also reduced in fasting,[12] protein restriction,[13,14] poorly controlled diabetes mellitus,[6] osteoporosis,[15] and hepatic failure.[16] It is increased in acromegaly,[11] renal failure,[11] and the third trimester of pregnancy.[11]

The role of IGFBP-3 in the injury response has not been characterized. The carrier function of IGFBP-3 suggests a long-term way of delivering IGF to wounds by using IGFBP-3 as a carrier for IGF-1. An IGF-1:IGFBP-3 complex was found to accelerate wound healing better than IGF-1 alone.[17] In a group of six fasting, critically ill surgical patients 3 to 18 days after admission to intensive care, a marked reduction in IGFBP-3 was reported in comparison to a normal-serum pool sample of healthy adults.[18] No attempts had been made to investigate the role of the GH–IGF-1–IGFBP-3 axis in the early catabolic flow phase of severe injury. We have previously reported a significant acute deficiency of plasma GH[19] and IGF-1[20] in trauma victims.

Since circulating IGFBPs have the potential to influence biopotency of IGF-1, we considered it of interest to investigate the correlation between plasma IGF-1 concentration and IGFBP-3 level in the early flow phase of severe injury in trauma victims.

SUBJECTS AND METHODS

Patients

Sixteen patients (13 men and three women) aged 19 to 84 years (mean, 47) with multiple injuries (Injury Severity Score, 21 to 50) and 16 healthy volunteers (Table 1) provided informed consent for this study, which was approved by our Institutional Review Board. At the time of study, none of the patients were septic or had multiple organ failure, diabetes, recent weight loss, or liver, renal, or malignant disease. There was no evidence of prior malnutrition. All multiple-injury patients required mechanical ventilatory support from the beginning of the study. Clinical characteristics of the patients are listed in Table 1. The majority of patients (12 of 16) were involved in motor vehicle crashes and had associated bone

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Table 1. Subject Characteristics (mean ± SEM)

Characteristic	Trauma Patients (13 men and 3 women)	Normal Controls (6 men and 10 women)
Weight (kg)	80.4 ± 3.6	75.1 ± 5.4
Age (yr)	47 ± 7	38 ± 3
BMI (kg/m²)	26 ± 2	28 ± 3
ISS	32 ± 2	—
REE		
%BEE	130 ± 5	—
kcal/d	2,256 ± 147	—
kcal/kg/d	27.8 ± 1.8	—
RQ	0.74 ± 0.02	—
N loss (mg/kg/d)	185 ± 15	—

Abbreviations: BMI, body mass index; ISS, Injury Severity Score; RQ, respiratory quotient.

fractures with extensive soft tissue damage. Three patients had multiple penetrating wounds to the abdomen, face, or chest. One patient was admitted with a closed head injury and multiple fractures after his experimental aircraft crashed into a tree. These multiple-trauma victims were hypermetabolic and highly catabolic.

Clinical stabilization was achieved in the acute stage of injury, during which patients received replacement and maintenance fluids with electrolytes but no calories or nitrogen. Forty-eight to 60 hours after injury when the ebb/shock phase had subsided, a morning (8 AM) blood sample was drawn for hormonal analysis. All patients had indwelling urinary catheters, and a 24-hour urine sample was collected in plastic bottles containing bactericidal sodium fluoride tablets and kept in an ice bath. Resting energy expenditure (REE) was measured by indirect calorimetry with a Horizon Metabolic Cart (Sensormedics, Anaheim, CA) from breath analysis of oxygen consumption and carbon dioxide production rates. The index of hypermetabolism was then estimated by dividing the patient's measured REE by basal energy expenditure (BEE) as calculated from the Harris-Benedict equation.

Postabsorptive morning blood samples (uninjured normal control) were obtained from 16 adult volunteers after overnight fasting. These controls were recruited from laboratory and office personnel, and all were in good health and consuming a normal diet without any medication. No dietary or activity restriction was imposed on uninjured control subjects during this period.

Analytic Methods

Plasma IGF-1 level was measured in duplicate after acid-ethanol extraction from its carrier proteins using a kit from Nichols Institute Diagnostics (San Juan Capistrano, CA). The technique involves separation of soluble IGF-1 from binding proteins that are precipitated with 87.5% ethanol and 12.5% 2N HCl. This method is simpler and less labor-intensive than the column extraction procedure. Sensitivity of the assay was 0.06 ng/mL, and precision (intraassay variance) was 3%. Insulin and hGH levels were also measured using respective radioimmunoassay kits. A sensitive and specific competition protein–binding radioimmunoassay kit (catalog #40-6055) developed by Nichols Institute Diagnostics was used for plasma IGFBP-3 assay. During incubation, radiolabeled IGFBP-3 competed with unlabeled IGFBP-3 in the test sample, standards, and controls for a limited number of specific antibody binding sites. At the end of the incubation period, antibody-bound IGFBP-3 was separated from free IGFBP-3 using antirabbit (donkey)-coated cellulose in suspension as the solid phase. After a brief incubation and centrifugation, unbound IGFBP-3 was aspirated and the antibody-bound radiolabeled ^{125}I-IGFBP-3 was measured in a gamma counter. A standard curve was prepared using a dose-response relationship, and the test sample and control concentrations were read from the curve. Samples were analyzed in duplicate after 1:100 dilution. The lowest measurable concentration in a sample was 50 ng/mL (assay sensitivity). The coefficient of intraassay variation was 5%.

Total nitrogen level in excreted urine was measured using a Chemiluminescence Digital Nitrogen Analyzer (Antek Instruments, Houston, TX). All group data are presented as the mean ± SEM. Statistical significance ($P \leq .05$) between groups was ascertained using an unpaired Student's t test. Linear correlations by the least-squares method were obtained between IGFBP-3–dependent parameters.

RESULTS

These polytrauma patients (Table 1) were hypermetabolic (REE 30% > BEE) and highly catabolic (losing N 15 g/d) and required mechanical ventilation. In this early catabolic flow phase of severe injury, anabolic IGF-1, GH, and IGFBP-3 levels were significantly ($P < .05$) decreased as compared with postabsorptive normal levels, perhaps as an adaptive measure (Table 2). These subnormal IGF-1 and GH levels confirm our previous findings[19,20] in similar groups of patients. Figure 1 illustrates the significant inverse relationship between age and IGFBP-3 levels in normal controls and trauma patients. There is also a significant positive relationship between IGF-1 and IGFBP-3 levels in normal controls and trauma patients (Fig 2). A correlation could not be established between IGFBP-3 levels and either the Injury Severity Score, REE/BEE, body mass index, GH, or insulin. The ratio of IGFBP-3 to IGF-1 levels, a possible indicator of inverse growth response,[21] is 16.1 ± 0.9 in our normal controls, which is not changed (18.7 ± 3.2) due to severe injury. This is compared with a reported ratio of 23 in girls with Turner's syndrome, which was reduced to 12 due to GH treatment.[21]

DISCUSSION

IGFBPs are high-affinity, soluble carriers of IGF-I and IGF-II that are present ubiquitously in extracellular fluid. Human fibroblasts synthesize and secrete some IGFBP-3 along with IGFBP-4. Binding to IGFBPs is required for IGF-1 to stimulate fibroblasts to secrete IGFBPs.[22] IGFBPs control IGF transport, efflux from the vascular compartment, and association of IGF with cell-surface receptors, and modify cellular response to growth factors. Protein binding prolongs the metabolic half-life of IGF-1 in the circulation and serves as a metabolic reservoir. The fact that IGFBP-3 is the major carrier of IGF-1 probably accounts for the stability of IGF-1 in blood. Understanding the role of IGFBPs in critical illness is essential because they control the availability of IGF to tissues.

Table 2. Hormone Levels in Patients and Controls

Hormone	Trauma Patients	Normal Controls
IGF-1 (ng/mL)	100 ± 18*	184 ± 12
IGFBP-3 (ng/mL)	1,590 ± 123*	2,885 ± 205
IGFBP-3/IGF-1	18.7 ± 3.2	16.1 ± 0.9
GH (ng/mL)	1.16 ± 0.20*	2.31 ± 0.33
Insulin (μIU/mL)	11.1 ± 1.2	10.4 ± 3.9

*$P < .05$ (v normal controls).

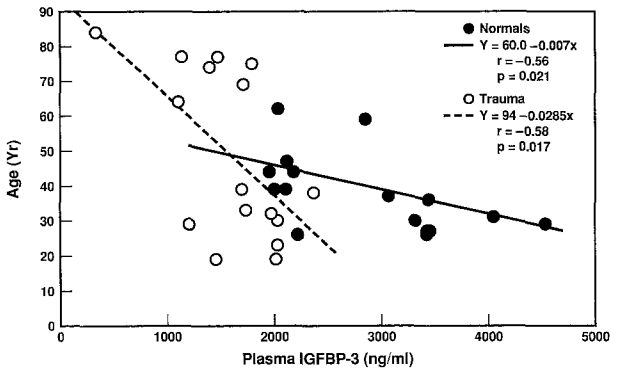

Fig 1. Inverse correlation between plasma IGFBP-3 levels (x axis, ng/mL) and age (y axis, years) in normal controls and trauma victims.

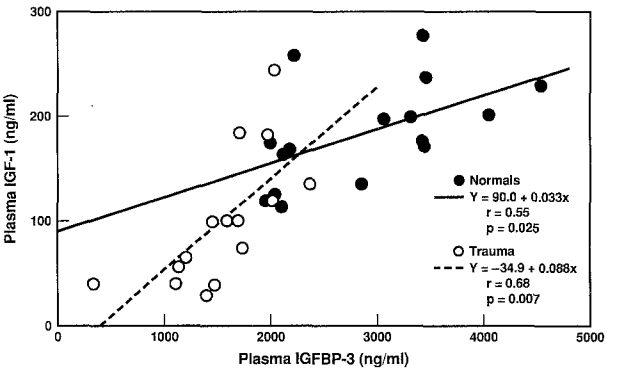

Fig 2. Positive correlation between plasma IGFBP-3 (x axis, ng/mL) and IGF-1 (y axis, ng/mL) levels in normal controls and trauma victims.

Plasma levels of IGFBP-3 are 16-fold greater than plasma IGF-1 levels in uninjured normals. This ratio is not altered significantly in the catabolic phase of acute injury, although their absolute levels, as well as GH levels, were decreased by 50%. It has been shown that diminished GH secretion or action via GH receptors, as in hypopituitarism and Laron dwarfism, causes a decrease in circulating IGF-1, which in turn causes a decrease in the GH-dependent IGFBP-3.[4] Elevations of GH levels, in the presence of functional GH receptors, produce elevations of serum IGF-1 and IGFBP-3 levels and lead to a large circulatory pool of IGFs. Our results support this model of the GH–IGF-1–IGFBP axis that is closely regulated by the metabolic status of the individual, which thereby controls bioavailability of IGF-1. There is a significant linear relationship (Fig 2) between plasma levels of IGF-1 and IGFBP-3 in controls, as well as in injured subjects. The nature of the relationship seems to indicate that there may exist saturation kinetics at the higher concentration of the protein.

IGFBP-3 may inhibit or potentiate IGF action. The mechanism by which IGFs are transferred from the circulatory pool to tissue receptors is at present unclear. IGFBP-3 has a strong tendency to associate with the cell surface, which decreases its affinity for IGF-1, promoting release of IGF-1 from an active complex.[23] Enzymatic proteolysis of IGFBP-3 by an IGFBP-3–specific protease present in plasma during pregnancy,[24] during severe illness,[18,25] after surgery,[26,27] and in diabetes[28] may facilitate release of IGF-1, although it does not disrupt the complex per se. The presence of this circulating protease may be an adaptive response to increase the bioavailability of IGFs and possibly to enhance nitrogen retention and counter the catabolic state in severe injury. Since the specific protease activity was not measured in this study, the possibility exists that the IGFBP-3 level measured may partially represent proteolytic fragments and not intact IGFBP-3. Demonstration of specific protease activity for IGFBP-3 in the injured state may be a valuable addition to understanding the regulatory mechanism in the bioavailability of circulating IGF-1.

Plasma GH,[19,29] IGF-1,[20,30] and IGFBP-3[9] levels are reported to decline consistently with advancing age in healthy subjects. Aging seems to be associated with changes in both the production and peripheral utilization of these hormones. Similar trends are also seen in the catabolic phase of severely injured trauma victims.[19,20] The present study confirms the changes in GH and IGF-1 levels in injured subjects and also reports a linear inverse relationship between IGFBP-3 and advancing age (Fig 1). Our results may have significant clinical implications if GH therapy is to be considered beneficial for the elderly. Increasing serum concentrations of GH would potentially change the conservation of IGF-1 in the circulation, delivery of IGF-1 to target tissues, and biologic effectiveness of the peptide. Measurement of serum IGFBP-3 changes induced by GH in trauma patients would be a major priority in understanding the therapeutic efficacy of adjuvant GH.

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