Radioimmunoassay for Insulin-Like Growth Factor II (IGF-II)

KUMIKO ASAKAWA, NAOMI HIZUKA, KAZUE TAKANO, IZUMI FUKUDA, IZUMI SUKEGAWA, HIROSHI DEMURA AND KAZUO SHIZUME

Department of Internal Medicine, Institute of Clinical Endocrinology
Tokyo Women’s Medical College and
Research Laboratory, The Foundation for Growth Science,
Tokyo, 162, Japan

Abstract

Insulin-like growth factor II (IGF-II) levels in human plasma were measured in physiological and pathological conditions by radioimmunoassay (RIA) with biosynthetic IGF-II. This RIA was specific for IGF-II and cross-reactivity with IGF-I was 1%. The sensitivity was 15 pg/tube with 50% displacement at 50 pg/tube. The intra- and inter-assay coefficients of variation for IGF-II were 6.3 and 9.3%, respectively.

The plasma IGF-II levels in normal adults, patients with hypopituitarism and patients with active acromegaly were 589.6 ± 15.8, 800.9 ± 45.6 and 330.3 ± 24.3 ng/ml, respectively. After human growth hormone (hGH) treatment in hypopituitarism, IGF-II slightly increased, but not significantly. After adenomectomy in patients with acromegaly, IGF-II significantly decreased. These data indicate that IGF-II concentrations in plasma were partially GH dependent. The GH dependency was less than that of IGF-I. IGF-II was low in patients with anorexia nervosa and with liver cirrhosis and high in patients with renal failure. In two cases with extrapancreatic tumor-associated hypoglycemia, plasma IGF-II was increased to 1123.8 and 843.5 ng/ml, and returned to normal after tumor resection.

These data showed that IGF-II was partly dependent on GH and nutritional conditions and that IGF-II was the most likely cause of some cases of hypoglycemia with extrapancreatic tumor. This specific and sensitive RIA of IGF-II would be useful in evaluating its physiological and pathological role in plasma and tissue.

Two main forms of IGFs have been isolated from human plasma and these two IGFs are structurally similar (Rinderknecht and Hummel, 1976, 1978). Because of the presence of these two IGFs, efforts have been made to develop methods which specifically measure these peptides. Radioimmunoassay for IGF-II is now available in several laboratories (Moses et al., 1980; Zapf et al., 1981; Daughaday et al., 1981; Hintz et al., 1982; Enberg et al., 1984). Recently IGF-II was synthesized by re-
combinant DNA technology. In this study we used biosynthetic IGF-II for RIA of IGF-II, measured plasma IGF-II levels in physiological and pathological conditions in man with this sensitive and specific RIA and compared these results with data from other laboratories.

Materials and Methods

Peptides

Biosynthetic human IGF-II (lot No. T51-ZL7-92) and porcine insulin were kindly provided by Eli Lilly Co. (Indianapolis, IN). Biosynthetic human IGF-I (lot No. 102981K) was donated by Fujisawa Pharmaceutical Co. (Osaka, Japan). Monoclonal antibody to rat IGF-II was provided by Amano Pharmaceutical Co. (Nagoya, Japan) (Tanaka et al., 1985).

Iodination of IGF-II

IGF-II was iodinated to a specific activity of 4.44–5.18 MBq/μg by a modification of the chloramine T method (Zapf et al., 1981). The reaction products were separated on a 0.7×50 cm Sephadex G-50 column.

RIA for IGF-II

Radioimmunoassay was carried out at 4°C in 0.03 M phosphate buffer, pH 7.5, containing 0.25% bovine serum albumin, 0.02% sodium azide, 0.02% protamine sulphate, and 0.01 M EDTA. IGF-II standard or unknown samples (50 μl) were preincubated with anti-IGF-II antibody (50 μl) for 72 h in a total volume of 0.45 ml. At the end of preincubation, 125I-IGF-II (50 μl: 10000 cpm) was added and incubated for 16–18 h. Separation of bound from unbound IGF-II was performed by the double antibody method.

Subjects

Forty-eight healthy adult volunteers (24 males and 24 females, 24–50 yr) and 36 normal children (25 males and 11 females, 0–19 yr) were studied to determine the normal range of plasma IGF-II. Plasma IGF-II concentrations in 28 patients with active acromegaly (20 males and 8 females), 24 patients with hypopituitarism, 9 with Turner’s syndrome, 7 with liver cirrhosis, 7 with renal failure, 16 with diabetes mellitus, 12 with anorexia nervosa, and 30 pregnant woman were measured. Another two patients with tumor-associated hypoglycemia (Suzuki et al. 1989; Haruki et al. 1990) were studied to measure IGF-II values in plasma and tumor extract. One patient had mesothelioma in the abdomen and the other patient had histiocytoma in the postperitoneum. IRI concentrations were both low and in the former case the tumor contained a large amount of IGF-II mRNA.

The patients with active acromegaly had increased plasma GH that was not suppressed below 5 ng/ml by the oral administration of glucose (75 g), and/or had paradoxical GH response to TRH and/or GnRH. In the untreated hypopituitarism, the diagnosis of GH deficiency was established by the failure of peak plasma GH to rise above 5 ng/ml after provocative stimuli such as insulin-induced hypoglycemia. They have been treated with the appropriate replacement therapy for other deficient pituitary hormones. The diagnosis for liver cirrhosis, renal failure and diabetes mellitus was established by clinical and laboratory findings.

EDTA-plasma was taken from each subject and stored at -20°C until the assay. EDTA-plasma was extracted by the acid-ethanol extraction method (Daughaday et al., 1980, 1987). Tumor tissue was extracted with aceton-formic acid (Russel et al., 1989).

Gel filtration of plasma at neutral pH

One milliliter of EDTA plasma was chromatographed on a 1.5×90 cm column of Sephacryl S-200 with 0.1 M phosphate buffer containing 0.1 M NaCl. One-milliliter aliquots were collected, and lyophilized. They were extracted with acid-ethanol and assayed for IGF-I (Miyakawa et al. 1986) and IGF-II.

Results

Characteristics of RIA for IGF-II

Figure 1 shows the standard curve of the RIA for IGF-II and cross-reactivity of the antibody with insulin and IGFs. The sensitivity of the RIA was 15 pg/tube with 50% displacement at 50 pg/tube. No cross-reactivity of the antibody with insulin.
IGF-II RIA

Fig. 1. Comparison of the dose response curves for human IGF-II, human IGF-I, insulin and acid-ethanol extracted plasma.

was observed. IGF-I crossreacted with it at a potency 100 times less than IGF-II. The intra- and inter-assay coefficients of variation of the assay were 6.3% and 9.3%.

The dilution curve for the acid-ethanol extract of human plasma were parallel to that for the IGF-II standard (Fig. 1).

**Plasma IGF-II concentrations**

Extracted IGF-II concentrations in 48 normal adults ranged from 389.4 to 845.4 ng/ml with a mean of $589.6 \pm 15.8$ ng/ml (Mean±SEM) as shown in Fig. 2. There was no difference between IGF-II concentrations in females and those in males ($601.2 \pm 22.7$ vs $578.0 \pm 22.4$ ng/ml). In normal children, plasma IGF-II was low in infants but the IGF-II concentrations in 5-year-olds were almost same as those of normal adults (Fig. 3).

In 20 patients with hypopituitarism plasma IGF-II ranged from 155.4 to 576.7 with a mean of $330.3 \pm 24.3$ ng/ml, which was statistically lower than in normal subjects. However, the IGF-II concentrations

Fig. 2. Plasma IGF-II in normal adults and patients with hypopituitarism or acromegaly.
in patients with hypopituitarism overlapped with those in normal subjects. Six pituitary dwarfs had received hGH treatment for several years. Plasma IGF-II concentrations before and after treatment were 389.1 ± 50.8 ng/ml and 440.5 ± 67.6 ng/ml. The IGF-II concentrations after treatment were slightly higher than those before treatment, but there was no significant difference between these two groups. In 28 active acromegalic patients plasma IGF-II ranged from 535.5 to 1350.0 with a mean of 800.9 ± 45.6 ng/ml (Fig. 2). The IGF-II values in acromegalic patients were statistically greater than those in normal subjects, but they overlapped. Thirteen acromegalic patients underwent operations for tumor resection. IGF-II was 644.0 ± 49.0 ng/ml after treatment, which was statistically different from before treatment (768 ± 62.2 ng/ml). However, in 6 of 13 patients IGF-II did not decrease after treatment.

Plasma IGF-II concentrations in patients with liver cirrhosis and anorexia nervosa were 273.7 ± 61.1 ng/ml and 342.7 ± 40.9 ng/ml.
ng/ml, respectively and these values were lower than those of normal subjects (Fig. 4). Six patients with anorexia nervosa received intravenous hyperalimentation therapy (Table 1). In three patients (cases 1, 2, and 3), whose body weight increased, IGF-II was high after treatment. However, in two other patients (cases 4 and 5) whose body weight increased less than 2 kg and one patient (case 6) who had a long history of emaciation, IGF-II did not change. In patients with renal failure plasma IGF-II was $831.5 \pm 119.1$ ng/ml, which was significantly higher than in normal subjects. In pregnant women there was no difference in IGF-II in first, second and third trimesters ($604.8 \pm 33.6$, $601.3 \pm 31.5$ and $654.0 \pm 31.9$ ng/ml). IGF-II in patients with diabetes mellitus or Turner's syndrome did not differ from that of normal subjects (Fig. 4).

The plasma IGF-I and IGF-II concentrations in two patients with extrapancreatic tumor associated hypoglycemia were measured. Before tumor resection, plasma IGF-II was 1123.8 and 843.5 ng/ml and
plasma IGF-I was 41.2 and \(<35\text{ ng/ml}\), respectively. After tumor resection, hypoglycemia disappeared. IGF-II concentrations were normalized to 555.4 and 422.1 ng/ml and IGF-I was normalized to 143.1 and 96.4 ng/ml (Fig. 5). IGF-II values in the tumor were 14.2 and 12.9 \(\mu\text{g/g tissue}\).

**Gel filtration pattern of normal plasma**

The Sephacryl S-200 gel filtration patterns of IGF-I and IGF-II were observed (Fig. 6). There were two peaks of both IGFs, approximately 150K and 40K. The 40K peak of IGF-II was higher than that of IGF-I.

**Discussion**

In the present study we reported IGF-II concentrations in man in physiological and pathological conditions determined by a specific and sensitive radioimmunoassay with biosynthetic IGF-II. This antibody to IGF-II was specific for IGF-II and cross reactivity with IGF-I was 1%. Zapf *et al.* (1981) and Enberg *et al.* (1984) reported that their polyclonal antibody to human IGF-II exhibited 10% cross-reactivity with IGF-I and their RIAs required correction for IGF-I content. Our RIA did not require it. Fifty percent displacement of \(^{125}\text{I}-\text{IGF-II}\) in our RIA was 50 pg/tube (100 pg/ml) while it was 1–2 ng/ml in other RIAs. Therefore, we could detect even a very small amount of IGF-II in various samples with this assay.

Plasma IGF-II in patients with active acromegaly was increased, and then decreased after operation. Plasma IGF-II in hypopituitarism was low. When these patients were treated with hGH, IGF-II increased slightly, but not significantly. Our data suggest that IGF-II might be partially GH dependent, but this GH dependency is less than that of IGF-I. It has been reported that no increase in IGF-II was found in patients with acromegaly, whereas GH deficiency was accompanied by significantly lower concentrations (Zapf *et al.* 1981, Enberg *et al.*, 1984). Hintz *et al.* (1982) showed that in GH deficient patients their IGF-II increased with acute and chronic GH treatment. They also suggested that IGF-II is partly GH dependent. However, our data differ from theirs. Zapf *et al.* have shown that the mean IGF-II values in acromegalic patients were the same as those in normal subjects, but in their data there were several patients with a higher concentration of IGF-II. Therefore, we suspect that some patients with acromegaly might have higher IGF-II than normal. The data for patients with anorexia nervosa suggest that the IGF-II concentration depends on nutritional conditions, but this dependency is also less than that of IGF-I. These results suggest that the IGF-II concentration in human plasma might be partially dependent on GH and/or nutritional conditions, and this dependency is less than that of IGF-I. The main control for plasma IGF-II needs to be clarified.

Our data concerning liver diseases, renal failure, and pregnant women are in accordance with those of other laboratories.

In patients with tumor associated hypoglycemia, plasma IGF-II increased slightly and IGF-I was low, and IGF-II in the tumor was high. After tumor resection, IGF-I and II were back to normal. We suspect that hypoglycemia is due to IGF-II that was produced by the tumor. There have been several reports of similar cases in which the tumor secretes IGF-II and hypoglycemia occurs (Megyesi *et al.* 1974, Hyodo *et al.*, 1977, Daughaday *et al.*, 1981, 1988, Shapiro 1988). In these cases, the immunoreactive plasma IGF-II concentration was normal or increased slightly and the tumor had increased concentration of IGF-II and IGF-II mRNA. Daughaday *et al.* (1980) reported that a tumor secreted high
molecular weight IGF-II and that a considerable loss of high molecular weight IGF-II may have occurred during the acid-ethanol extraction process, and a loss of IGF-II may have occurred in storage. They also showed that in these cases IGF-II was higher in the radioreceptor assay than in radioimmunoassay. Therefore the reason why the immunoreactive plasma IGF-II concentration was not extremely high might be the loss of IGF-II and the molecular heterogeneity of IGF-II. It is interesting to study the molecular weight, receptor reactivity and bioactivity of IGF-II in our cases. There would be many patients with tumor-associated hypoglycemia and we could determine the cause in some patients by means of the assay of IGFs.

With this sensitive and specific RIA of IGF-II with biosynthetic IGF-II we were able to measure IGF-II in human plasma and might measure small amounts of IGF-II in tissue or cerebrospinal fluid, which is usable in evaluating the role of IGF-II.

Acknowledgements

We are greatly indebted to Eli Lilly Co. (Indianapolis, IN) for supplying biosynthetic IGF-II and insulin, Fujisawa Pharmaceutical Co. (Osaka, Japan) for biosynthetic IGF-I, and Amano Pharmaceutical Co. (Nagoya, Japan) for monoclonal antibody to IGF-II. We thank Dr. M. Iwashita, Dr. Y. Suzuki and Dr. K. Haruki for supplying samples from pregnant patients and patients with extrapancreatic tumor with hypoglycemia.

This work was partly supported by Grants in Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (Nos. 63770872 and 01770878), and Yoshioka Memorial Fund.

References


