Clin Pediatr Endocrinol 1993;2(Suppl 2):79-84

Insulin-Like Growth Factor (IGF)-II and IGF-Binding Protein in Human Spinal Fluid

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Abstract. Insulin-like growth factor-II (IGF-II) and its specific receptor are present in the central nervous system (CNS) of experimental animal models, where this peptide has unique roles in neuronal growth and differentiation. IGF-II in the cerebrospinal fluid (CSF), as well as in plasma, is bound to specific binding protein (IGFBP). In this study we examined CSF IGF-II levels and IGFBP patterns in children with various physiological and pathological states. IGF-II, dissociated from IGFBP by acid gel chromatography, was measured by RIA, and IGFBP analyzed by western-ligand blotting. CSF IGF-II levels were 16.7±4.9 ng/ml in control subjects and there were no age-related variations. In some cases with CNS tumor, hydrocephalus and inflammatory conditions, their CSF IGF-II levels were increased. CSF IGFBP mainly consisted of 34kd, 31kd and 24kd protein. In contrast to serum IGFBP, CSF IGFBP patterns were almost unaffected by age, and were increased in some CNS inflammatory conditions. Although the specific role of IGF-II in the human CNS remains to be defined, our results suggest that the regulatory mechanisms for the IGF-II/IGFBP system are present in the human CNS as well as in animal models.

Key words: insulin-like growth factor II, insulin-like growth factor binding protein, cerebrospinal fluid, central nervous system

Introduction

Insulin-like growth factor-II (IGF-II) and its specific receptor have been found in the fetal and adult central nervous system (CNS) of animal models [1,2,3]. The specific roles of this receptor-ligand system in the CNS remain to be defined, although evidence is accumulating which suggests that IGF-II might participate in prenatal growth, differentiation and development of the CNS [4]. IGF-II is also present in human brain extracts and spinal fluid (CSF) [5,6]. IGF-II in CSF, as well as in plasma, is bound to specific binding protein (IGFBP), which has been shown to have a higher affinity for IGF-II than for IGF-I [7] and is supposed to be involved in the modulation of IGF actions. In this study we examined the IGF-II levels and IGFBP patterns of human children's CSF in various physiological and pathological conditions.

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Materials and Methods

Sample collection

CSF specimens were collected from 35 control subjects aged from two months to 15 years, and from patients with leukemia receiving intrathecal chemotherapy (12 cases), meningeal leukemia (3 cases), medulloblastoma (1 case), CNS hemorrhage (4 cases), congenital hydrocephalus due to toxoplasmosis (1 case), viral encephalitis (4 cases), Reye syndrome (1 case), and Guillain-Barre syndrome (2 cases). Samples were immediately frozen and stored at −20℃ until assay.

Gel chromatography

CSF sample 0.5 ml, acidified with 30 μl acetic acid, was applied to Superose HR10/30 column (Pharmacia) equilibrated with 1M acetic acid containing 150 mM NaCl and 0.02 % NaN_3 at a flow rate of 0.5 ml/min. 0.5 ml fractions were collected, and the fractions corresponding to free [^{125}I]IGF-II were lyophilized and dissolved in RIA buffer.

Radioimmunoassay (RIA) for IGF-II

IGF-II levels were determined by RIA according to the method of Lee by using anti-IGF-II monoclonal antibody (Amano) [8]. Figure 1 shows the IGF-II standard curve and the cross-reactivity of IGF-I and insulin.

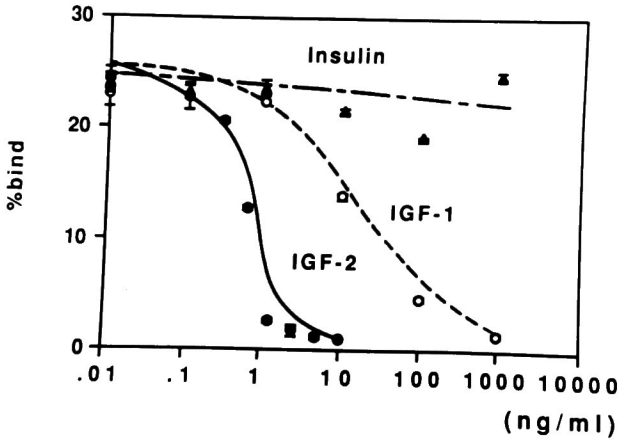


Fig 1. Standard curve for IGF-II and cross reactivity of IGF-I and insulin in IGF-II RIA system.

Western ligand blotting of CSF IGFBP

CSF samples were concentrated 5 times by Mizubutorikun^R (Atto). Western ligand blotting was performed as previously described using [^{125}I]IGF-II [7].

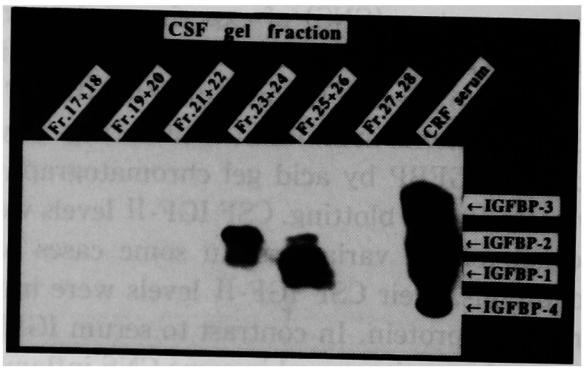


Fig 2-a. Western ligand blotting of CSF IGFBP in acid-gel fractions and serum IGFBP pattern of chronic renal failure.

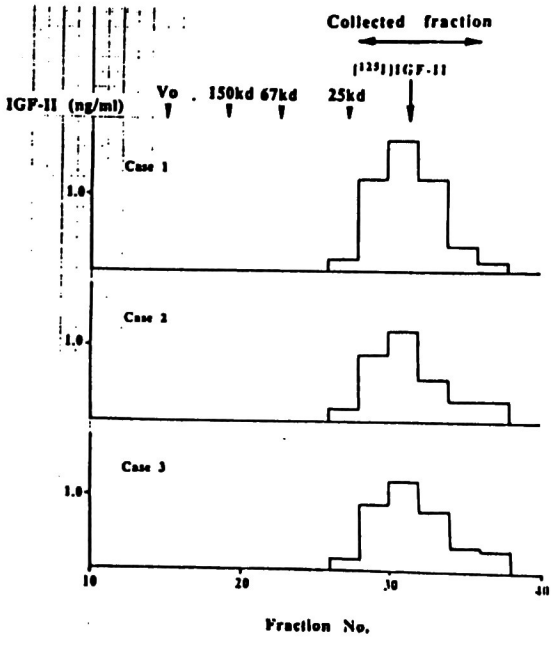


Fig 2-b. IGF-II contents in each gel fraction.

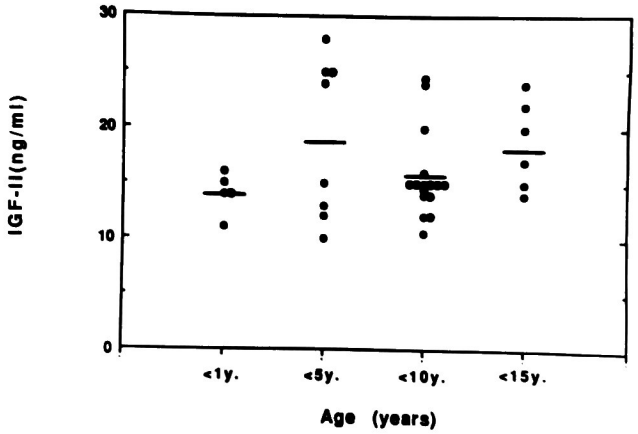


Fig 3. CSF IGF-II levels in each age group.

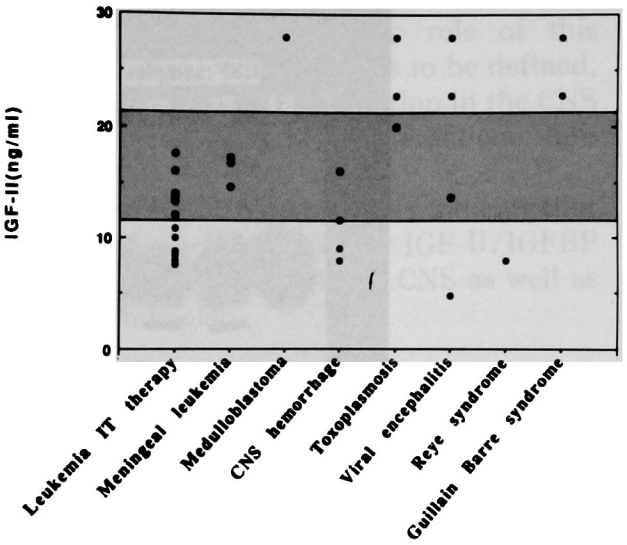


Fig 4. CSF IGF-II levels in various CNS disease conditions. Shaded area indicates the mean±SD of control subjects.

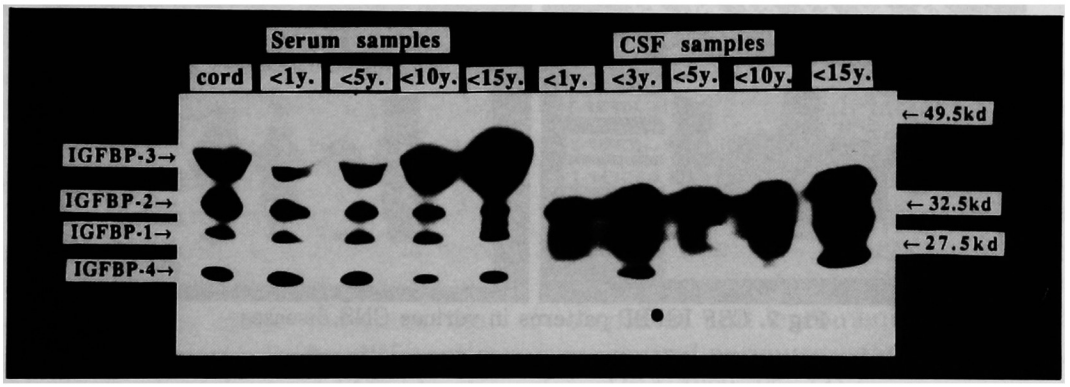


Fig 5. Western ligand blotting of serum and CSF IGFBP in each age group.

Results

CSF IGFBP was detected in fraction No. 23−26 (Figure 2-a), and IGF-II could be dissociated from its binding protein by acid gel chromatography (Figure 2-b).

There were no age-related variations in CSF IGF-II levels, which were 16.7±4.9 ng/ml over all (Figure 3).

Figure 4 shows CSF IGF-II levels in various disease conditions. In some cases of medulloblastoma, congenital toxoplasmosis, viral encephalitis and Guillain-Barre syndrome, CSF IGF-II levels were elevated.

CSF IGFBP mainly consisted of 34kd, 31kd and 24kd protein, which correspond to IGFBP-2, -1 and -4 respectively (Figure 5). In contrast to serum IGFBP, CSF IGFBP patterns were almost unaffected by age. CSF IGFBP, especially IGFBP-2 and -1, revealed a higher affinity for IGF-II than for IGF-I (Figure 6).

In medulloblastoma, CNS inflammation, hemorrhage and congenital toxoplasmosis,

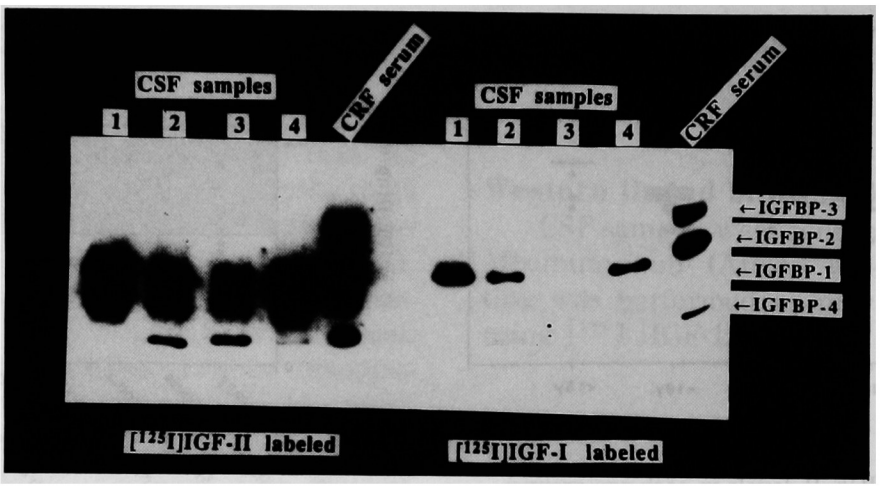


Fig 6. Western ligand blotting of CSF and chronic renal failure serum IGFBP labeled with[125]IGF-II and [125] IGF-I.

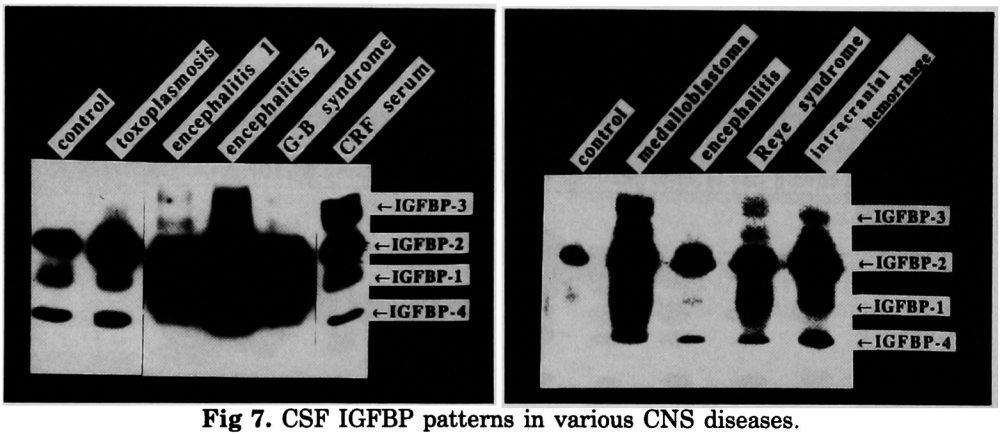
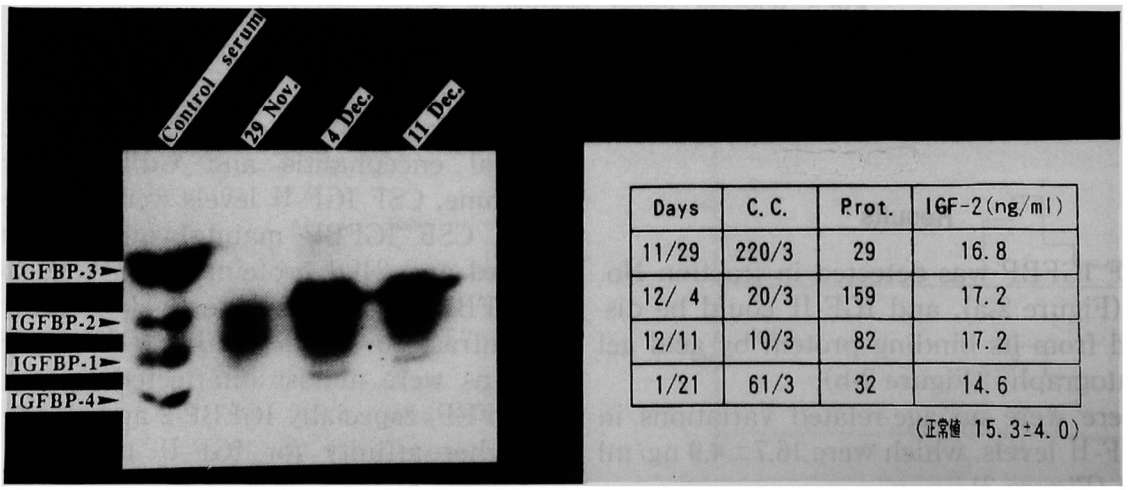


Fig 7. CSF IGFBP patterns in various CNS diseases.



Days	C.C.	Prot.	IGF-2(ng/ml)
11/29	220/3	29	16.8
12/ 4	20/3	159	17.2
12/11	10/3	82	17.2
1/21	61/3	32	14.6

(正常値 15.3±4.0)

Fig 8. Serial changes of CSF IGFBP (figure) and IGF-II levels (table) in the case of meningeal leukemia.

CSF IGFBP-2 and -1 were quantitatively increased, and IGFBP-3, normally not detected in control CSF, was also increased in some of these cases (Figure 7). Serial examinations of the case of meningeal leukemia revealed that his CSF-IGFBPs were increased, and there were no significant changes in CSF IGF-II levels (Figure 8).

Discussion

Immunoreactive IGF-II was found in human CSF at a concentration of 16.7±4.9 ng/ml without any age-related changes, which values were lower than those reported by Haselbacher, 30.6±9.5 ng/ml [6]. This discrepancy was partially explained by the differences in the RIA system and IGF-II extraction method. The source of CSF IGF-II remains to be defined. Previous reports suggest that CSF IGF-II/IGF-I ratios were much higher than those of serum samples [6]. These data may indicate that CSF IGF-II was locally produced in the CNS. In fact, IGF-II is present in human brain extracts [5], and IGF-II mRNA is synthesized throughout the rat CNS [1,2]. In our study, CSF IGF-II levels were increased in some CNS disease conditions. Although the previous study indicates that IGF-II may have some roles in the differentiation and development of the fetal CNS [4], the physiological and pathological significance of CSF IGF-II in the adult CNS remains to be elucidated.

There were three main IGFBP in CSF, which showed a higher affinity for IGF-II than for IGF-I as previously described [7], and no significant age-related variations. This CSF IGFBP pattern was definitely different from serum IGFBP. In experimental animal models, neuronal cells, astrocytes and choroid plexus have been shown to produce IGFBP [9,10,11]. In addition to the role in IGF transport, these IGFBP regulate tissue response to IGF by potentiating or inhibiting the biological action of IGF at target tissue [11,12]. In our study, CSF IGFBPs were quantitatively increased in some CNS disease states. Although the specific role of this IGFBP in human CNS remains to be defined, an understanding of IGF-II action in the CNS must take IGF-II/IGFBP interactions into account.

Our results in this study may indicate that the regulatory mechanisms for IGF-II/IGFBP systems are present in human CNS as well as in animal models.

Acknowledgement

This work was supported by a research grant from the Foundation for Growth Science in Japan.

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