Insulin-like growth factors and IGF binding proteins in cyst fluid from patients with craniopharyngioma prior to intracavitary irradiation with ⁹⁰Yttrium and thereafter

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Abstract

Aim—To examine a series of cyst fluid samples from patients with craniopharyngioma at various stages of treatment in order to evaluate the use of insulin-like growth factors (IGFs) and IGF binding proteins as tumour markers or indicators of successful treatment, or both.

Methods—Cyst fluid samples were obtained by stereotactic puncture prior to the intracavitary application of ⁹⁰Yttrium and at subsequent occasions. Analysis was performed by gel chromatography, radio-immunoassays, binding studies, and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with subsequent western blotting.

Results—IGF-I, -II and IGF binding protein-1 concentrations were measured in three craniopharyngioma cyst fluid samples. Immunoreactive IGF-I and IGF binding protein-1 concentrations in these three samples were between 6 and 29 ng/ml, and 17 and 48 ng/ml, respectively. In contrast, the IGF-II concentrations measured in 19 cyst fluid samples from seven patients with craniopharyngioma at various stages of treatment were much higher at 25–671 ng/ml. SDS-PAGE and subsequent western blotting using [¹²⁵I]IGF-II as the ligand gave bands with estimated molecular weights of 330, 220, 135, 96, 46, 43, 34, 29, and 13.5 kDa in one adult, and identical bands at 220, 41.5, 37.5, 32, and 19 kDa in three cyst fluid samples from three children with craniopharyngioma.

Conclusions—These results suggest that IGFs and IGF binding proteins are secreted by craniopharyngiomas and that they may alter the growth characteristics of these tumours. Furthermore, the distinct pattern of IGF binding protein sizes might be used as a tool for the differential diagnosis of tumours of the central nervous system.

(J Clin Pathol: Mol Pathol 1996;49:M51-M56)

Keywords: craniopharyngioma, insulin-like growth factors, IGF binding proteins, tumour marker.

Craniopharyngiomas account for about 9% of all primary childhood brain tumours with a particular high incidence around the age of eight years.¹ Their location is predominantly

suprasellar, either in the pituitary stalk or hypothalamus. It is thought that they arise from Rathke's pouch where high levels of insulin-like growth factor (IGF) II mRNA expression have been detected.²

Cyst fluid from malignant brain tumours contains various growth factors, including IGF, that are mitogenic for various cell lines.³⁻⁶ Inhibitory factors such as IGF binding proteins have also been identified in cyst fluid.⁷

IGF-I and -II are mitogenic polypeptides and are synthesised by various tissues. In brain, IGF-I is widely distributed,⁸ while IGF-II expression is limited to the plexus choroideus, leptomeninges and the fetal Rathke's pouch.^{2,9-12} IGFs are bound to specific IGF binding proteins which modulate the effect of IGFs on their target cells.¹³ Of these, IGF binding protein-2 is the predominant IGF binding protein species in both fetal and adult brain.¹⁴

The present study was undertaken to evaluate the presence of IGFs and the heterogeneity of IGF binding proteins in cyst fluid samples from patients with craniopharyngioma.

Methods

Cyst fluid samples were obtained by stereotactic puncture from seven patients (A-G) (age range two to 43 years) with craniopharyngioma prior to an intracavitary injection of ⁹⁰Yttrium¹⁵ (day 0) and at one to three succeeding punctures thereafter (range day 11 to day 1946).

ACID ETHANOL EXTRACTION

Cyst fluid samples (100 µl) were incubated with 400 µl acid ethanol (0.15% 12 M HCl) for 30 minutes. To neutralise the reaction, 200 µl 0.86 M Tris/HCl was added and the mixture was centrifuged at 3000 rpm for 20 minutes. An aliquot of 100 µl of the supernatant fluid was subsequently analysed by specific radio-immunoassays for IGF-I and IGF-II.

GEL FILTRATION

The cyst fluid samples (250 µl) were acidified with 250 µl 2 mol/l acetic acid and loaded onto a Sephacryl S-100 HR column (38 × 1 cm; Pharmacia, Uppsala, Sweden) equilibrated with 1 mol/l acetic acid (pH 4.0). V₀ is equivalent to a K_d value of 0 and V_i to a K_d of 1.

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Accepted for publication
5 October 1995

Figure 1 Elution profiles of IGF-I and -II after acid gel chromatography of craniopharyngioma cyst fluid samples from patients B, C and F ($V_o = K_d$ of 0 and $V_i = K_d$ of 1; peaks are labelled I, II and III).

Labelled IGF binding protein-1 is eluted at a K_d of 0.24 and IGF-I at a K_d of 0.66. IGFs were eluted and measured in the K_d range 0.47 to 0.77.

Immunoreactive IGF-I, IGF-II and IGF binding protein-1 concentrations (ng/ml) in cyst fluid samples from three patients with craniopharyngioma

Patient	Age (years)	IGF-I		IGF-II		IGF binding protein-1
		GC	AE	GC	US	US
B	6.6	29	460	404	730	48
C	15.8	6	420	25	800	38
F	18.2	17	320	330	220	17

GC=gel chromatography; AE=acid ethanol extraction; US=unseparated sample.

BINDING STUDIES

Ten microlitres of cyst fluid were preincubated with labelled des(1-3)-IGF-I, IGF-I or IGF-II and re-run on a Sephacryl S-100 HR column (29.5×1 cm) equilibrated with 0.05 mol/l phosphate buffer (pH 7.4). Each fraction was counted in a gamma counter and the results were expressed as the percentage of total radioactivity.

RADIOIMMUNOASSAYS

IGF-I and IGF-II concentrations were measured using specific radioimmunoassays as described previously.⁴ Briefly, polyclonal rabbit antibody (donated by Professor Gluckman, Auckland, New Zealand) directed against recombinant human IGF-I (KabiGen, Stockholm, Sweden) was used in the IGF-I radioimmunoassay (RIA). Des(1-3)-IGF-I was used as a ligand and recombinant hIGF-I as a standard. For IGF-II, a RIA with recombinant hIGF-II (KabiGen) as a standard and hen yolk antibody raised against hIGF-II was used. For IGF binding protein-1, a specific RIA was used as described in detail by Povia *et al.*¹⁶

WESTERN BLOTTING

Unreduced craniopharyngioma cyst fluid samples from one adult and three paediatric patients (B, C and F) were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12.6% polyacrylamide slab gel ($160 \times 105 \times 1.5$ mm) as described by Hossenlopp *et al.*¹⁷ The proteins were then transferred onto nitrocellulose sheets and subsequently incubated with [¹²⁵I]IGF-II. The dried nitrocellulose filter was then exposed at -70°C on a Hyperfilm-MP (Amersham, Little Chalfont, UK) for seven days.

Results

ELUTION PATTERN FOR IGF-I AND IGF-II

Figure 1 shows the elution profiles of IGF-I and IGF-II from three craniopharyngioma cyst fluid samples after acid gel filtration and specific radioimmunoassays. High immunoreactivity was observed for IGFs in the K_d range 0.0–0.3 (molecular weights >100 kDa); immunoreactivity was rather low in the K_d range 0.6–0.8 where free IGF-I and IGF-II are eluted. High immunoreactivity was also found in the K_d range 0.3–0.6 where the elution of IGF binding proteins occurs.

IGF-I, IGF-II AND IGF BINDING PROTEIN-1 CONCENTRATIONS

IGF-I, -II and IGF binding protein-1 concentrations were measured in three craniopharyngioma samples (table). Immunoreactive IGF-I and IGF binding protein-1 concentrations in three cyst fluid samples ranged between 6 and 29 ng/ml, and 17 and 48 ng/ml, respectively. IGF-II concentrations in two of three samples were about 20-fold higher than the respective IGF-I concentrations. As clearly demonstrated, it is crucial that separation of

Figure 2 Immunoreactive IGF-II concentrations were measured in a total of 19 cyst fluid samples from seven patients (A–G) before (1) intracavitary irradiation with ^{90}Y and at subsequent punctures thereafter (2–4): patient A, days 0, 18 and 271; patient B, days 0, 11, 32, and 449; patient C, days 0 and 1946; patient D, days 0 and 28; patient E, days 0, 130 and 144; patient F, days 0 and 131; patient G, days 0, 199 and 335.

IGFs from the IGF binding proteins is achieved by gel chromatography as determination of unseparated samples or samples extracted in acid ethanol may give falsely raised results.

Immunoreactive IGF-II concentrations were measured in a total of 19 cyst fluid samples from seven patients before and after intracavitary irradiation with ^{90}Y (fig 2). In most patients a decrease in IGF-II concentrations was noted after intracavitary irradiation. However, the extent and duration of the decrease varied greatly. In patient A, there was a slight increase in cyst fluid IGF-II concentration from 78 ng/ml at day 0 to 86 ng/ml at day 18; however, nine months after treatment the IGF-II concentration had fallen to 54 ng/ml. In patient B, there was a sharp decrease in IGF-II concentration from 557 to 56 ng/ml within 11 days of treatment. At day 32 the IGF-II concentration was 671 ng/ml and fell to 404 ng/ml at day 449. In patient C, there was a long term decrease in cyst fluid concentrations of IGF-II from 71 ng/ml at day 0 to 25 ng/ml at day 1946. In patient D, IGF-II concentrations fell from 64 ng/ml at day 0 to 45 ng/ml at day 28. In patient E, an initial increase in IGF-II concentrations from 73 ng/ml at day 0 to 322 ng/ml at day 130 was followed by a decrease to 54 ng/ml at day 144. In patient F, there was a clear decrease from 330 ng/ml at day 0 to 42 ng/ml at day 131. In patient G, IGF-II concentrations were 58, 51 and 76 ng/ml at days 0, 199 and 335, respectively.

In total, five of seven patients responded to treatment with a reduction in their cyst fluid IGF-II concentrations. In the remaining two patients a decrease in IGF-II concentrations was observed at a subsequent puncture. In two patients the decrease in IGF-II concentrations did not persist and a further increase was observed at subsequent punctures.

BINDING STUDIES

Chromatography of cyst fluid samples from two patients after preincubation with labelled IGFs yielded three different peaks (fig 3) with K_d ranges of 0.03–0.09 (peak I), 0.2–0.4 (peak II) and 0.6–0.8 (peak III). IGF-I, des(1–3)-IGF-I and IGF-II bound more strongly at peak II than at peak I. When unlabelled IGFs were added, peak II could be displaced and shifted to peak III, increasing the amount of free IGFs. Surprisingly, des(1–3)-IGF-I showed significant affinity to IGF binding proteins and unlabelled peptide could block this binding specifically.

WESTERN BLOTTING

SDS-PAGE and western blotting, using [^{125}I]IGF-II as a ligand, were carried out on craniopharyngioma cyst fluid samples from one adult and three paediatric patients (B, C and F). Autoradiography revealed bands corresponding to the following molecular weights: 330, 220, 135, 96, 46, 43, 34, 29, and 13.5 kDa in the adult and 200, 41.5, 37.5, 32, and 19 kDa in the paediatric patients (fig 4).

Discussion

The present study demonstrates the presence of IGFs and IGF binding proteins in the cyst fluid samples from patients with craniopharyngioma.

In addition to our previous results,⁴ there is evidence for the presence of a binding protein capable of binding the truncated form of IGF-I. This IGF-I variant lacks three amino acids at the N-terminus and has virtually no affinity for IGF binding proteins.¹⁸ The binding of des(1–3)-IGF-I is specific as the addition of unlabelled peptide could displace the binding of this truncated IGF-I to the IGF binding protein. The displacement is not absolute, probably due to the addition of insufficient amounts of competitor, but it seems that binding of des(1–3)IGF-I is specific and that it is due to IGF binding proteins with molecular weights of 30–50 kDa and not to IGF receptor proteins, which are eluted at a lower K_d (peak I). However, this does not preclude the possibility that smaller cleavage products of the IGF-II receptor are responsible for binding of des(1–3)-IGF-I. Further evaluation of this observation is clearly warranted.

The concentration of IGF-II was up to 20-fold higher than the respective IGF-I concentrations. Furthermore, the IGF-II concentrations in the craniopharyngioma cyst fluid samples were significantly higher than those found in astrocytoma and prolactinoma cyst fluid samples.^{5,6} It is possible that craniopharyngioma cells synthesise considerable amounts of IGF-II as high levels of IGF-II mRNA expression have been found in the epithelial components of Rathke's pouch in the rat fetus.² Moreover, fetal brain expresses higher levels of IGF-II mRNA compared with adult brain, where expression is restricted to the choroid plexus and leptomeninges.^{10,19,20} We suggest that measurement of the concentration

Figure 3 Gel chromatography at neutral pH of two cyst fluid samples preincubated with $[^{125}\text{I}]\text{trIGF-I}$, $[^{125}\text{I}]\text{IGF-I}$ or $[^{125}\text{I}]\text{IGF-II}$ with or without unlabeled peptide. $V_0 = K_d$ of 0 and $V_i = K_d$ of 1; peaks are labelled I, II and III.

of IGF-II in craniopharyngioma cyst fluid may be useful for the differential diagnosis of other brain tumour cysts—for example, prolactinoma and glioma.

Although the concentration of immunoreactive IGF binding protein-1 in these cysts is low, craniopharyngiomas seem to synthesise other IGF binding proteins in considerable abundance. Preliminary immunohistochemical data showed weak staining for IGF binding protein-2 and IGF binding protein-5, strong staining for IGF binding protein-3 and none for IGF binding protein-1 (Zumkeller *et al*, unpublished data, 1995). IGF binding protein-2 seems to be the predominant IGF binding protein in the central nervous system and its level of expression in the fetal rat brain stem, cerebral cortex and hypothalamus is much higher than that of IGF binding protein-1.²¹ Expression of IGF binding protein-2 mRNA

has also been found in the choroid plexus of adult rats.²² Thus, the observed low immunoreactivity for IGF binding protein-1 in our cyst fluid samples concurs with the immunohistochemical data. These results also indicate that various other IGF binding proteins are secreted by craniopharyngiomas.

The IGF binding protein pattern on western blotting differs in cyst fluid samples from adults and children with craniopharyngiomas. The bands were identical in all three paediatric cyst fluid samples, with a major band at 37.5 kDa. In the adult craniopharyngioma cyst fluid sample, the major band was at 46/43 kDa, possibly IGF binding protein-3. Bands at 45, 40 and 36.5 kDa were also described in cultured rat astroglial and neuronal cells.^{9,23} A 34 kDa band was also found in astrocytoma⁵ and prolactinoma cyst fluid samples,⁶ which could be identical with the one detected in cerebrospinal

Figure 4 SDS-PAGE and western blotting of craniopharyngioma cyst fluid samples from an adult (A) and three children (B) using labelled IGF-II (lanes A–C, patients B, C and F, respectively). Low and high molecular weight markers (Pharmacia) were used.

fluid and human fetal cerebral cortex.²⁴ Malignant gliomas synthesise an IGF binding protein with a molecular weight of 35 kDa (shown to be IGF binding protein-2) in addition to a 24 kDa form²⁵ which was not present in our samples. We have also found bands at 19 and 13.5 kDa, which have not been described before but fetal neurons have been reported to synthesise a 23 kDa IGF binding protein,¹⁴ which might be related to our 19 kDa form.

The high molecular weight bands at 330, 220, 200, 135, and 96 kDa may represent subunits, truncated forms, monomers, or dimers of IGF receptors. The 220 and 200 kDa bands may reflect a truncated form of the type II IGF receptor whereas the 135 and 96 kDa bands may represent type I IGF receptor subunits.²⁶ The IGF-II receptor is widely distributed in the pituitary and choroid plexus of fetal and adult rats.²⁷ Bands of this size are consistently found in the cyst fluid of other brain tumours.^{5,6}

In conclusion, stereotactic intracavitary irradiation with ⁹⁰Yttrium or ¹⁸⁶Rhenium has been widely carried out in patients with cystic craniopharyngiomas.^{15,29} A reduction in the content of IGF-II in the cyst fluid after intracavitary irradiation may indicate a good re-

sponse to such a treatment. Monitoring the cyst fluid IGF-II concentrations may provide an additional parameter with which to judge the success of treatment.

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