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

W Zumkeller, M Säaf, T Rähn

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, radioimmunoassays, 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.


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

Cranio-pharyngiomas 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.3-9 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, while IGF-II expression is limited to the plexus choroides, leptomeninges and the fetal Rathke’s pouch.2-12 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 radioimmunoassays 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 x 1 cm; Pharmacia, Uppsala, Sweden) equilibrated with 1 mol/l acetic acid (pH 4.0). V0 is equivalent to a Kd value of 0 and Vt to a Kd of 1.
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 x 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. 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 Povoa 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 12.5% polyacrylamide slab gel (160 x 105 x 1.5 mm) as described by Hossenlopp et al. 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 Kd range 0-0-0-3 (molecular weights >100 kDa); immunoreactivity was rather low in the Kd range 0-6-0-8 where free IGF-I and IGF-II are eluted. High immunoreactivity was also found in the Kd 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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>IGF-I</th>
<th>IGF-II</th>
<th>IGF binding protein-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>6-6</td>
<td>29</td>
<td>404</td>
<td>460</td>
</tr>
<tr>
<td>C</td>
<td>15-8</td>
<td>6</td>
<td>25</td>
<td>420</td>
</tr>
<tr>
<td>F</td>
<td>18-2</td>
<td>17</td>
<td>330</td>
<td>320</td>
</tr>
</tbody>
</table>

GC = gel chromatography; AE = acid ethanol extraction; US = unseparated sample.
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 (A-G) before (1) intracavitary irradiation with 188Yttrium and at subsequent punctures thereafter (2-4): patient A, days 0, 11, and 32; patient B, days 0, 11, 32, and 449; patient C, days 0, 1946; patient D, days 0 and 58; patient E, days 0, 130 and 144, patient F, days 0 and 131; patient G, days 0, 199 and 335.

**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 188Yttrium and at subsequent punctures thereafter (2-4): patient A, days 0, 11, and 32; patient B, days 0, 11, 32, and 449; patient C, days 0 and 1946; patient D, days 0 and 58; patient E, days 0, 130 and 144, patient F, days 0 and 131; patient G, days 0, 199 and 335.

**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 Kd (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 prolatinoma cyst fluid samples.76 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.101920 We suggest that measurement of the concentration...
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.23 A 34 kDa band was also found in astrocytoma and prolactinoma cyst fluid samples,24 which could be identical with the one detected in cerebrospinal fluid.

Figure 3 Gel chromatography at neutral pH of two cyst fluid samples preincubated with [125I]trIGF-I, [125I]IGF-I or [125I]IGF-II with or without unlabelled peptide. $V_c = K_c$ of 0 and $V_l = K_l$ of 1; peaks are labelled I, II and III.
IGFs and IGF binding proteins in the cyst fluid

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. 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 form 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, 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.

The IGF-II receptor is widely distributed in the pituitary and choroid plexus of fetal and adult rats. Bands of this size are consistently found in the cyst fluid of other brain tumours.

In conclusion, stereotactic intracavitary irradiation with 90Yttrium or 186Rhenium has been widely carried out in patients with cystic craniopharyngiomas. A reduction in the content of IGF-II in the cyst fluid after intracavitary irradiation may indicate a good response to such a treatment. Monitoring the cyst fluid IGF-II concentrations may provide an additional parameter with which to judge the success of treatment.

10 Hynes MA, Brooks PJ, Van Wyk JJ, Lund PK. Insulin-like growth factor II messenger ribonucleic acids are syn-