Macroprolactinaemia associated with prolactin adenoma

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BACKGROUND: Macroprolactinaemia, defined as hyperprolactinaemia with a predominance of, or only, the big big prolactin (bbPRL) isoform, is considered idiopathic and poorly symptomatic. Since its association with a PRL adenoma is poorly documented, we examined a series of 13 patients with tumoral hyperprolactinaemia for the presence of macroprolactinaemia. METHODS: From a series of 36 patients with hyperprolactinaemia studied for PRL isoforms, we selected 13 with hyperprolactinaemia and a prolactinoma, and divided them into two groups on the basis of the predominant PRL isoform, the large PRL group (five patients), with a predominance of the big PRL isoform, and the monomeric PRL (mPRL) group (eight patients), with a predominance of the mPRL isoform. Plasma PRL concentrations were measured by radioimmunoassay, while plasma PRL heterogeneity was studied by gel filtration chromatography. The plasma autoantibody-bound PRL and the histology of the tumours were also studied. RESULTS: Macroprolactinaemia was seen in five out of the 13 patients with a PRL adenoma. The clinical and biological characteristics of the groups with and without macroprolactinaemia were similar. In the large PRL group, no evidence for anti-PRL autoantibodies was found and the prolactinomas were either typical or exhibited unusual aggregates of immunoreactive PRL deposits, the latter suggesting the tumoral origin of these large forms. CONCLUSION: Our results suggest that PRL adenoma may be associated with macroprolactinaemia.

Key words: hyperprolactinaemia/macroprolactinaemia/pituitary tumours/prolactinomas

Introduction

After the isolation of the 23 kDa monomeric form of prolactin (mPRL), which is considered to be the biologically active form, two other forms with different molecular weights, big PRL (50 kDa; bPRL) and big big PRL (150 kDa; bbPRL), were identified by gel filtration chromatography of serum or extracts of normal pituitary gland and pituitary tumours (Suh and Frantz, 1974; Fang and Refetoff, 1978; Sinha et al., 1984; Allolio et al., 1987; Fonseca et al., 1991). Gel filtration is a relatively insensitive technique, requiring high levels of prolactin, so it is difficult to study these PRL isoforms in normal subjects with low plasma PRL levels, especially since the big and big big forms represent only 25–30% of the total PRL immunoreactivity (Blacker et al., 1994).

In the 1980s, a new type of hyperprolactinaemia, termed macroprolactinaemia, was identified (Whittaker et al., 1981; Andersen et al., 1982; Andino et al., 1985; Jackson et al., 1985; Larrea et al., 1985; Corenblum, 1990; Carlson et al., 1992; Hattori et al., 1992a) and found to occur in 8–25% of patients with hyperprolactinaemia (Hattori et al., 1992a; Bjoro et al., 1995; Hattori, 1996; Olukoga and Kane, 1999; Leslie et al., 2001; Valette-Kasic et al., 2002). This entity was defined by the bbPRL isoform being the only, or the predominant, form, and was claimed to be poorly symptomatic and idiopathic. Although the nature of these large forms is still under debate (reviewed in Fraser and Lun, 1990), a tumoral origin has been suggested (Rogol and Rosen, 1974; Ohnami et al., 1987).

The identification of a prolactinoma in three hyperprolactinaemic patients with predominantly the bbPRL isoform prompted us to study these large forms in patients with tumoral hyperprolactinaemia and to evaluate the clinical, biological and histological characteristics of these patients.

Subjects and methods

Subjects

From a series of 36 patients with hyperprolactinaemia in whom we studied PRL isoforms between 1994 and 1996, we selected 13 (11 women and two men, aged 18–38 years) who presented with hyperprolactinaemia (plasma PRL levels >30 μg/l) and a prolactinoma: tumour was either proven by histology and immunocytochemistry (eight patients) or was highly probable on the basis of the combination of plasma PRL levels >100 μg/l and a microadenoma detected by pituitary magnetic resonance imaging (five patients). These patients were referred to the Endocrinology Departments at the Lyon and St Etienne hospitals.

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Six women presented with amenorrhoea associated with galactorrhoea; three were taking a combined estrogen–progestogen contraceptive pill and the symptoms persisted despite treatment being halted. Of the remaining five women, three presented with amenorrhoea that had developed over 6 months after 2 years of spaniomenorrhoea, while the other two presented with galactorrhoea alone. None of the 11 women were on antidepressive drugs. There were no clinical, hormonal, and/or ultrasonographic features of polycystic ovarian syndrome and none had hypothyroidism. All had normal body weights (body mass index >20 kg/m²).

One man presented with gynaecomastia and the other with infertility associated with oligoasthenozoospermia on the spermogram.

All patients were treated with bromocriptine; because of side-effects, eight (six women and two men) underwent transphenoidal surgery.

**Prolactin studies**

**Prolactin assay**

Blood samples, collected between 10:00 and 12:00, were centrifuged and the plasma stored at −20°C until assayed. Plasma PRL concentrations were measured by radioimmunoassay using a rabbit anti-human PRL antibody prepared in our laboratory (Claustrat et al., 1994). The results were expressed as µg/l by comparison with standard MRC 75/504.

**Chromatography**

Plasma PRL heterogeneity was studied by gel filtration chromatography. Plasma samples (<2 ml, depending on the PRL concentration) were applied to a Sephadex G100 column (120 cm × 2 cm) and eluted using 0.05 mol/l phosphate buffer, pH 7.5, containing 0.1% bovine serum albumin (BSA). The column was calibrated with Blue dextran, free 125I, and [125I]PRL (rat PRL does not react with the antihuman PRL antiserum). Thirty fractions were collected, their PRL concentrations determined by radioimmunoassay, and the percentage of each form calculated.

**Detection of autoantibody-bound PRL**

This was performed as described by Hattori et al. (1992b). Briefly, plasma samples (100 µl) and [125I]PRL (100 µl, 10 000 cpm) diluted in phosphate-buffered saline (0.05 mol/l, pH 7.4) containing 0.5% BSA were incubated overnight at 4°C, then 200 µl of 25% (w/w) polyethylene glycol (PEG 6000; Merck, France) was added, the mixture centrifuged, and theradioactivity in the precipitate measured in a γ-counter. Non-specific binding was determined using 20 plasma samples from different normal subjects as controls in each series. A plasma was considered positive when the amount of radioactivity precipitated differed significantly from that precipitated by the controls, using Student’s t-test (confidence interval of 95%).

**Tumour studies**

Eight PRL adenomas were studied by light microscopy and immunocytochemistry. For light microscopy, pieces of tumour tissue were immersed in Bouin–Hollande fixative for 4 days, then embedded in paraffin. Sections (5 µm thick) were prepared and stained using the Herlant’s tetrachrome and periodic acid–Schiff–orange G methods. Amyloid deposits were detected using Congo red.

The tumour type was identified immunocytochemically. Serial sections were processed by the indirect immunoperoxidase method using a streptavidin–biotin complex (Dako A/S, Denmark), as previously described (Trouillas and Girod, 1996). Mouse monoclonal antibodies against human proteins (indicated by h), obtained from Immunotech, Marseille, France [anti-hPRL (164-22-12), anti-βhLH (300-10-E-14-3), and anti-α-subunit (326-2-1; lot f 1079)] and from Dako A/S, Copenhagen, Denmark (anti-βhTSH) and rabbit polyclonal antibodies (anti-hGH and anti-βhLH; both kindly donated by Dr A.F.Parlow, National Institute of Arthritis, Diabetes and Digestive and Kidney Disease, Bethesda MD, USA) were used at dilutions of 1/200 to 1/10 000.

**Results**

The main data, including the clinical features and PRL levels and heterogeneity are summarized in Table I.

### Table I. Clinical features and prolactin (PRL) levels in 13 patients with tumoral hyperprolactinaemia

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical data</th>
<th>Total PRL (µg/l)</th>
<th>mPRL (µg/l)</th>
<th>bPRL (%)</th>
<th>bbPRL (%)</th>
<th>Adenoma size (mm)</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large PRL group</td>
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<td></td>
<td></td>
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<tr>
<td>1</td>
<td>F</td>
<td>24</td>
<td>G</td>
<td>198</td>
<td>2 (1)</td>
<td>1</td>
<td>98</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>30</td>
<td>I</td>
<td>303</td>
<td>76 (25)</td>
<td>16</td>
<td>59</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>35</td>
<td>G</td>
<td>202</td>
<td>91 (45)</td>
<td>1</td>
<td>54</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>18</td>
<td>A+G</td>
<td>115</td>
<td>35 (30)</td>
<td>7</td>
<td>63</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>38</td>
<td>A+G</td>
<td>201</td>
<td>38 (19)</td>
<td>13</td>
<td>68</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>Monomeric PRL group</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>6</td>
<td>F</td>
<td>30</td>
<td>A+G</td>
<td>126</td>
<td>71 (56)</td>
<td>37</td>
<td>7</td>
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<td>–</td>
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<tr>
<td>7</td>
<td>F</td>
<td>22</td>
<td>A</td>
<td>102</td>
<td>61 (59)</td>
<td>27</td>
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<td>6</td>
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</tr>
<tr>
<td>8</td>
<td>F</td>
<td>25</td>
<td>A</td>
<td>709</td>
<td>425 (60)</td>
<td>32</td>
<td>8</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>24</td>
<td>A+G</td>
<td>103</td>
<td>64 (62)</td>
<td>32</td>
<td>6</td>
<td>5</td>
<td>+</td>
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<tr>
<td>10</td>
<td>F</td>
<td>33</td>
<td>A</td>
<td>1539</td>
<td>1000 (65)</td>
<td>28</td>
<td>7</td>
<td>13</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>32</td>
<td>GynM</td>
<td>955</td>
<td>764 (80)</td>
<td>16</td>
<td>4</td>
<td>14</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>24</td>
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<td>110</td>
<td>95 (85)</td>
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<td>1</td>
<td>14</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>24</td>
<td>A+G</td>
<td>100</td>
<td>94 (94)</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>–</td>
</tr>
</tbody>
</table>

m = monomeric; b = big; bb = big big; A = amenorrhoea; G = galactorrhoea; I = infertility; GynM = gynaecomastia. Surgery: yes: +, no: –.
one man), the bbPRL isoform (Figure 1B) predominated. No evidence for anti-PRL autoantibodies was found in the large PRL group.

**Correlations between PRL isoforms, clinical symptoms and PRL adenomas**

All patients had clinical symptoms of PRL excess. In the mPRL group, all seven women presented with menstrual disturbances, associated with galactorrhoea in four. In the large PRL group, two women presented with menstrual disturbances associated with galactorrhoea and the two other with galactorrhoea alone. The man presented with infertility.

Total PRL levels were high in both the monomeric and large PRL groups (>100 µg/l). mPRL levels were high in all patients, except for one case in the large PRL group, a woman with a galactorrhoea without menstrual disturbances and with a low mPRL level (2 µg/l).

**Histological features of prolactinomas**

Histology and immunocytochemistry confirmed the presence of a PRL adenoma in all eight patients tested (four women and one man from the mPRL group and two women and one man from the large PRL group).

In all five patients with predominantly the mPRL isoform (nos. 8, 9, 10, 11 and 12) and in the male patient with predominantly the large PRL isoform (no. 2), the pituitary tumour exhibited the typical features of a sparsely granulated prolactinoma with a solid architectural pattern and numerous vessels. On immunocytochemical testing, all the cells were strongly stained with anti-hPRL antibodies. Bound antibody was located near the nucleus in a dot-like pattern, corresponding to the Golgi complex (Figure 2A). In the two women in the large PRL isoform group, a typical prolactinoma with irregular limits surrounded by non-tumoral pituitary with PRL cell hyperplasia was seen in one (no. 4), the large cells, arranged in wide rows, being strongly and diffusely PRL-positive, while, in the other (no. 5), the PRL immunoreactivity was unusual, with irregular aggregates of PRL deposits scattered in the cytoplasm of many cells, giving them a ‘clotted’ appearance (Figure 2B). In all tumours, all other antibodies tested gave negative results and no amyloid deposits were observed.

**Discussion**

Twenty years ago, macroprolactinaemia was first described as hyperprolactinaemia with exclusively, or a predominance of, the bbPRL isoform. This entity was defined as a hyperprolactinaemia that was poorly symptomatic (Andino et al., 1985; Jackson et al., 1985; Larrea et al., 1985) and idiopathic (Allolio et al., 1987; Malarkey et al., 1988). For several years, macroprolactinaemia did not attract much attention as the identity of the large forms was unknown and their identification by gel filtration difficult, expensive, and not current practice. However, over the last 3 years, the subject has been studied, especially since the description of the polyethylene glycol (PEG) method for the precipitation of large serum proteins (Fahie-Wilson, 1999; Olukoga and Kane, 1999; Leslie et al., 2001). Most studies have examined the frequency and the clinical and biological features of these macroprolactinaemias (Olukoga and Kane, 1999; Leslie et al., 2001; Valette-Kasic et al., 2002). No study has evaluated their association with a...
Prolactinoma in a population with macroprolactinaemia. In the series of 106 patients with macroprolactinaemia studied by Valette-Kasic et al. (2002), four cases of PRL adenoma (4%) were identified and proven by histology. Three adenomas were found by pituitary imaging in another series (Olukoga and Kane, 1999), but none was proven by histology.

The biological activity of these large forms seems to depend on the experimental model used. A lower biological activity of the large forms has been suggested as the reason for the lack of typical symptoms of hyperprolactinaemia; this is supported by in-vitro studies using the radioreceptor assay (Garnier et al., 1978; Farkouh et al., 1979) and, in some cases, using the N2b lymphoma model (Jackson et al., 1985; Larrea et al., 1989). Other studies using the N2b lymphoma model showed the in-vitro bioactivity of the small and large forms to be similar (Andersen et al., 1982; Rowe et al., 1983; Whitaker et al., 1984); in this case, the lack of symptoms cannot be explained in terms of lower bioactivity of the large forms. It is likely that, because of their high molecular weight, the large forms do not readily cross the capillary walls (Andersen et al., 1982; Larrea et al., 1985). All 13 patients reported here presented with clinical symptoms of PRL excess. The high plasma mPRL levels may explain the appearance of symptoms in both the large and monomeric PRL groups, as suggested by other authors (Jeske et al., 2002). In our study, one patient in the large PRL group did not fit this hypothesis, but the PRL adenoma was not confirmed by histology. The biological characteristics of the PRL adenomas associated with macroprolactinaemia were the same as those in patients with a predominance of the mPRL isoform. Levels of total PRL were high (≥100 µg/l), suggesting a tumoral hyperprolactinaemia.

The large forms seem to be heterogeneous in terms of aetiology, and the origin of these large forms remains controversial. For a long time, they were thought to be aggregates of mPRL. More recently, Hattori et al. (1992a, 1994) claimed that bbPRL was an anti-PRL autoantibody, but we did not find any evidence for autoantibodies in our patients with exclusively bbPRL. Two studies have reported an association between a PRL adenoma and macroprolactinaemia (Rogol and Rosen, 1974; Ohnami et al., 1987). In our study, this association was confirmed by histology in all three patients tested. The unusual immunocytochemical feature of deposits of PRL aggregates in one of these (patient no. 5) may suggest the tumoral origin of the large forms of PRL. This finding is in agreement with those of Ohnami et al. (1987), who, on the basis of the similarity of the chromatographic PRL patterns of tumoral extracts and serum samples from eight patients, concluded that these large forms are possibly secreted by the tumour.

Our results suggest that PRL adenoma may be associated with macroprolactinaemia. The clinical and biological characteristics of prolactinomas associated or not associated with macroprolactinaemia are similar. The unusual immunocytochemical features seen in one case may suggest the tumoral origin of these large forms. These results should be confirmed by a study of a larger cohort with tumoral hyperprolactinaemia and a chromatographic PRL study on both tumoral extracts and serum samples.

Figure 2. Immunocytochemical features in prolactinomas. (A) Typical appearance, with immunoreactivity located near the nucleus (arrow). (B) Regular aggregates of prolactin (PRL) immunoreactive deposits, giving a clotted appearance (arrow). Immunoperoxidase reaction using anti-hPRL antibody (×1000).
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