Bromocriptine-induced Morphological Changes in Cultured Growth Hormone-producing Pituitary Adenoma Cells

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Abstract

Tumor tissues obtained from two patients with growth hormone-producing pituitary adenomas were cultured and treated with bromocriptine, then examined for morphological changes. Untreated tumor cells (controls) were morphologically well preserved and in terms of growth hormone secretion. Tumor cells exposed to bromocriptine for 7 days contained many vacuoles, which, after 14 days of exposure, grew larger and more numerous. These vacuoles appeared to be extensions of endoplasmic reticulum: some were connected to rough or smooth endoplasmic reticulum or, occasionally, to Golgi apparatus, and there were ribosomes on their surfaces. Bromocriptine apparently has cytocidal effects on growth hormone-producing pituitary adenoma in vitro.

Key words: growth hormone, acromegaly, pituitary adenoma, bromocriptine

Introduction

Bromocriptine (BC), a long-acting dopamine agonist, has been applied clinically against growth hormone (GH)-producing and prolactin (PRL)-producing pituitary adenomas. BC therapy inhibits PRL secretion by the latter, and this is often accompanied by radiological evidence of tumor reduction. However, the mechanism of this tumor reduction has not been clearly identified. In addition to inhibiting hormone secretion, BC may also suppress hormone synthesis, leading to degeneration and reduction of relevant organelles within tumor cells and thereby effecting tumor reduction. Alternatively, BC may have cytocidal effects on PRL-producing pituitary adenomas, inhibiting proliferation of tumor cells by an intracellular negative feedback mechanism and causing tumor reduction through a decrease in the number of tumor cells.

Although BC inhibits GH secretion, tumor reduction rarely occurs in GH-producing pituitary adenomas. However, it remains to be determined whether or not BC causes any microscopic morphological changes in these tumors. We treated cultured GH-producing pituitary adenomas with BC and examined them for microscopic morphological changes within the tumor cells.

Materials and Methods

Tumor tissues were obtained from two patients with GH-producing pituitary adenomas who had not undergone preoperative BC therapy. The tissues were cultured with an organ culture technique in which the medium contained BC at a concentration of 12.5 μg/ml.

Fragments of tumor tissue measuring about 1 mm were placed on gelatin sponges and cultured in 10 ml of Eagle's minimum essential medium. The cultures were then incubated at 37°C in a moist atmosphere of 5% CO₂ and 95% air. From the beginning, BC-treated and untreated control groups were cultured separately. The former were treated with BC for 7 or 14 days. On the 7th and 14th days the culture medium was subjected to radioimmunoassay for confirmation of GH secretion. The cultured tumor tissues were then fixed in glutaraldehyde, embedded in epon, and examined with light (toluidine blue stain) and electron microscopy.

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Results

The tumor cells of the control group were all well preserved with respect to both cell morphology (Fig. 1) and GH secretion (Table 1), and therefore this procedure was deemed suitable for investigating morphological changes caused by BC. PRL appeared at low concentrations in the control group and in the 7-day BC-treated group (Table 2). After 7 days of exposure to BC, many vacuoles appeared in the cytoplasm of the tumor cells (Fig. 2 left). The vacuoles were larger and more numerous after 14 days of exposure. They apparently consisted of expanded endoplasmic reticulum (ER), as some were connected to either rough or smooth ER and, sometimes, to Golgi apparatus. In addition, ribosomes were present on their surfaces (Fig. 2 right). There were no observable changes in the size, distribution, or number of intracellular secretory granules or lysosomes.

Discussion

BC therapy alone rarely reduces GH-producing pituitary adenomas. But, Wass et al.\textsuperscript{16} reported that tumor reduction did occur in two acromegalic patients who received BC therapy without irradiation, suggesting effectiveness of BC. Schwinn et al.\textsuperscript{13} treated 37 active acromegalic patients with BC for

Table 1 GH concentration in culture medium (ng/ml)

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<th>Case 1</th>
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<tr>
<td></td>
<td>Control</td>
<td>BC-treated</td>
<td>Control</td>
<td>BC-treated</td>
</tr>
<tr>
<td>7 days</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>38-120</td>
<td>48-128</td>
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<tr>
<td>14 days</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>5-90</td>
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Table 2 PRL concentration in culture medium (ng/ml)

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<tr>
<td></td>
<td>Control</td>
<td>BC-treated</td>
<td>Control</td>
<td>BC-treated</td>
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<tr>
<td>7 days</td>
<td>12.5</td>
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<td>14 days</td>
<td>2.8</td>
<td>&lt;1.0</td>
<td>5.0-9.2</td>
<td>&lt;1.0</td>
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Fig. 1 Electron micrograph of a 14-day-old control culture. There are no degenerative changes in the cytoplasm of the tumor cells. Bar = 2 μm. N: nucleus, M: mitochondrion, Ly: lysosome, sg: secretory granules.

Fig. 2 left: Electron micrograph of a 7-day-old culture in the BC-treated group. Many vacuoles are seen in the cytoplasm of the tumor cells. Bar = 5 μm. right: Electron micrograph of a 14-day-old culture in the BC-treated group. Some vacuoles have ribosomes on their surfaces (arrow). Bar = 0.5 μm. N: nucleus, M: mitochondrion, V: vacuole.

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periods of up to 4 years. Neither increases in sellar size nor visual field defects occurred during treatment, so they speculated that BC not only decreases the GH level but may also inhibit tumor growth.

Morphological changes have not been detected in GH-producing pituitary adenomas treated preoperatively with BC. However, the reported cases all involved short-term treatment. Therefore, it is unknown whether or not long-term treatment would result in morphological changes. Moreover, such factors as dosage and drug concentration in tumor tissues may have influenced the results of the reported investigations.

Many investigators have cultured pituitary adenomas. We previously reported that monolayer cultures of GH-producing pituitary adenomas exhibit many degenerative changes in the absence of any drug. Therefore, monolayer cultures are not suitable for investigating morphological changes. Organ cultures in which gelatin sponges are used adequately preserve the morphology and three-dimensional structure.

The morphological changes we observed were mainly cytoplasmic vacuolar alterations, which appeared within 7 days of treatment and became more dramatic by the 14th day. Ultrastructurally, the numerous, abnormally large vacuoles appeared to be extensions of ER. Others have observed the same morphological changes in PRL-producing pituitary adenomas but not in GH-producing pituitary adenomas. Anniko et al. observed only a widening of the Golgi zone in GH-producing adenomas after incubation with BC at a concentration of 1.0–0.001 μg/ml. We used a tenfold greater concentration of BC as well as longer exposure, which may account for the difference between our results and those of Anniko et al.

In summary, the tumor cells treated with BC underwent degenerative cytoplasmic changes. This suggests that BC may have some cytocidal effects on GH-producing pituitary adenomas.

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