Effects of Bromocriptine and Terguride on Cell Proliferation and Apoptosis in the Estrogen-stimulated Anterior Pituitary Gland of the Rat

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Abstract

The effects of bromocriptine and terguride on the estrogen-stimulated anterior pituitary gland of the female Wistar rat were investigated. Pituitary weight and serum prolactin (PRL) levels were reduced by treatment with bromocriptine or terguride. Immunohistological staining for proliferating cell nuclear antigen (PCNA) revealed that the PCNA labeling index of PRL-producing cells was significantly decreased by treatment with bromocriptine or terguride compared with untreated cells. The number of apoptotic cells analyzed by the terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate-biotin nick end labeling method was significantly increased in rats treated with bromocriptine or terguride. Suppression of cell proliferation and induction of apoptosis are important effects of bromocriptine and terguride in the treatment of prolactinomas and other hyperprolactinemias.

Key words: prolactinoma, proliferative rate, apoptosis, bromocriptine, terguride

Introduction

Pituitary dopamine (D2) receptor agonists, part of the family of ergot-derived drugs, are effective in the treatment of hyperprolactinemia or prolactinomas. Bromocriptine and lisuride are the most widely used of these drugs. The effect of bromocriptine on prolactinomas has been investigated by morphological analyses including immunohistological and electron microscope studies. Terguride, a 9,10-transdihydrogenated analogue of lisuride, is a newly developed drug used for the suppression of hyperprolactinemia and is thought to act as a partial agonist on D2 receptors. Bromocriptine and terguride also cause the regression of prolactinomas but the mechanisms of these effects are not known.

This study investigated the effects of bromocriptine and terguride in the estrogen-stimulated rat anterior pituitary gland by analyzing the relationship between proliferative cell index, apoptotic cell index, and the drug administered.

Materials and Methods

Twenty-two female Wistar rats (Clea Japan, Inc., Tokyo), 4 weeks of age and weighing 125-150 g, were housed with free access to tap water and standard pellet food. Eighteen rats received subcutaneous estradiol dipropionate injections (2.5 mg/0.5 ml/rat) weekly for 8 weeks. The other four rats received no estrogen treatment. Beginning 1 week after completion of estradiol injections, groups of six rats received bromocriptine (5 mg/kg) or terguride (1 mg/kg) orally once a day for 4 weeks. Six other rats which had received estrogen injections received no oral agents during this period. Serum specimens were collected from all rats 3 to 5 hours after the final dose of bromocriptine or terguride, including rats receiving neither. Then all rats were killed under anesthesia using pentobarbital sodium injections. The pituitary glands were removed and the wet weight of each gland was recorded. Tissues were fixed with 10% buffered formalin, embedded in paraffin, sectioned, and then stained with HE. Immunohistological studies used two series of sections deparaffinized in xylene and rehydrated
through a graded series of ethanol to phosphate buffered saline. Endogenous peroxidase activity was blocked by placement in 0.3% hydrogen peroxidase in methanol for 30 minutes. The series of sections stained for the proliferating cell nuclear antigen (PCNA) (anti-PCNA; DAXO, San Diego, Calif., U.S.A.) was placed in 0.01 M citrate buffer and heated three times for 3 minutes at 500 W in a microwave oven (modified method according to Shi et al. 31). These sections were then saturated with 5% normal goat serum for 30 minutes and incubated with anti-PCNA antibody diluted 1:100 at 4°C for 24 hours or with anti-rat prolactin (PRL) antibody (A539/R4H; UCB-Bioproducts, Sandown, N.H., U.S.A.) diluted 1:200 at room temperature for 1 hour. The antigen-antibody complex was visualized using the avidin-biotin-peroxidase complex (ABC) method. Meyer's hematoxylin was used as a nuclear counterstain.

Apoptotic cells were detected with an apoptosis kit (MBSTAIN Apoptosis kit; MBL, Nagoya) based on the terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridinetriphosphate (dUTP)-biotin nick end labeling (TUNEL) method. 15) The deparaffinized sections were incubated with proteinase K at 37°C for 30 minutes and immersed in TdT buffer at room temperature for 10 minutes. After removal of this buffer, the sections were immersed in TdT solution (TdT buffer 90 µl, biotin-dUTP 5 µl, TdT 5 µl) at 37°C for 1 hour. This solution was then removed and the sections were placed in TB buffer (300 mM sodium chloride, 30 mM sodium citrate) at room temperature for 15 minutes. The antigen-antibody complex was visualized using the ABC method. Meyer's hematoxylin was used as a nuclear counterstain.

The PCNA labeling index and apoptotic cell index were calculated for representative sections. More than 2000 cell nuclei of the anterior pituitary glands were counted in several viable areas of the same section. All comparisons between groups used analysis of variance tests. P values less than 0.05 were considered significant. All values are given as the mean ± SD.

Results

PRL-producing tumors were induced in all estrogen-stimulated rat anterior pituitary glands (Fig. 1). No necrosis was seen. One estrogen-treated rat died after 7 weeks of estrogen stimulation because of massive growth of the prolactinoma. Table 1 shows the results of analysis of the pituitary glands and serum PRL measurement. Estrogen treatment significantly increased the weight (p < 0.01) and serum PRL level (p < 0.05) compared to the untreated rats. Administration of bromocriptine significantly reduced the effect of estrogen on the weight of the pituitary gland (p < 0.05) and on the serum PRL level (p < 0.05). Similarly, terguride administration significantly reduced the effect on the pituitary gland (p < 0.01) and the serum PRL level (p < 0.05). The anterior pituitary glands of all estrogen-stimulated rats contained PCNA-positive cells (Fig. 2). Both bromocriptine and terguride significantly reduced (p < 0.01) the PCNA labeling index (Table 1). The pituitary glands from rats which received bromocriptine or terguride contained apoptotic cells which showed condensed nuclear chromatin within the nuclear membrane and shrinkage of cells reflecting condensation of the nucleus and cytoplasm (Fig. 3). Both bromocriptine and terguride caused a significant increase (p < 0.05) in the apoptosis index (Table 1).

Discussion

Long-term estrogen stimulation is well-known to induce pituitary neoplasms in rats. 2, 20, 31, 38, 39) Prolactinomas are induced over a period of 7 weeks by weekly 2.5 mg subcutaneous estradiol injections. 20) In our study, estradiol dipropionate was used to induce prolactinomas from the lactotrophic cells of the anterior pituitary gland. Lactotrophic cell proliferation is the initial event in the development of PRL-secreting neoplasms following estrogen stimulation. 3) Estrogen decreases dopamine uptake in the lactotrophic cell, 10) increases receptors for thyroid-stimulating hormone-releasing hormone, 4) and induces hyper-
Table 1  Effects of bromocriptine and terguride on the pituitary glands and serum prolactin (PRL) level

<table>
<thead>
<tr>
<th>Treatment (n)</th>
<th>Pituitary gland weight (mg)</th>
<th>Serum PRL level (ng/ml)</th>
<th>PCNA labeling index (%)</th>
<th>Apoptotic cell index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (4)</td>
<td>8.8 ± 0.1</td>
<td>8.0 ± 0.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Estrogen (5)</td>
<td>30.6 ± 6.2**</td>
<td>538 ± 332*</td>
<td>2.1 ± 0.4</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Estrogen + bromocriptine (6)</td>
<td>21.6 ± 4.2†</td>
<td>91.4 ± 61.7†</td>
<td>0.6 ± 0.2**</td>
<td>0.23 ± 0.03†</td>
</tr>
<tr>
<td>Estrogen + terguride (6)</td>
<td>17.7 ± 2.8‡</td>
<td>29.4 ± 5.8‡</td>
<td>0.7 ± 0.1†</td>
<td>0.26 ± 0.10‡</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01 compared to the untreated rats. †p < 0.05, ‡p < 0.01 compared to the estrogen-treated rats.

Dopamine agonists stimulate D2 receptors which cause the suppression of PRL secretion.30 Administration of a dopamine agonist induces marked cellular involution characterized by reduction of the cellular, cytoplasmic, nuclear, and nucleolar sizes, an increase in the nuclear-cytoplasmic ratio, and a decrease in cytoplasmic volume densities of endoplasmic reticulum and Golgi complexes.33 Ergot derivatives such as bromocriptine and terguride reduce serum PRL levels in humans as well as rats.8,10,17,23,26,27,35,40 Part of the effect of estrogen on lactotrophic cells is mediated through inhibition of the actions of dopamine.8,23,27 Bromocriptine and terguride bind with high affinity to dopamine receptors in the pituitary gland and significantly lower the serum PRL levels in lactating rats.27,30 The bioavailability of terguride (approximately 30%) is five-fold higher than that of bromocriptine (approximately 6%).24,32,37 The amounts of these agents administered in our study were chosen according to these bioavailability figures. Both bromocriptine and terguride caused significant decreases in serum PRL levels and pituitary weights. PRL suppression continues for at least 11 hours with the peak bromocriptine concentration occurring at 3 hours.34 We collected serum specimens at 3 to 5 hours following the last drug administration. One rat receiving bromocriptine failed to sustain a significant decrease in serum PRL level. The frequency of clinical bromocriptine resistance ranges from 5% to 18%.6,7,13,29 Prolactinomas resistant to bromocriptine therapy showed no evidence of cell shrinkage by electron microscopy but contained demonstrable D2 receptor messenger ribonucleic acid. A mechanism other than D2 receptor loss might be responsible for the resistance to bromocriptine therapy.22 In our study, the bromocriptine-resistant rat had reduced proliferative index and apoptosis index, but not to the same

Fig. 2 Photomicrograph showing proliferating cell nuclear antigen-positive cells in the prolactinoma of the estrogen-stimulated rat. × 400.

Fig. 3 Photomicrographs showing apoptotic cells (arrow) in prolactinoma treated with bromocriptine (left) and terguride (right). The apoptotic cells show condensed nuclear chromatin within the nuclear membrane and shrinkage of cells reflecting condensation of the nucleus and cytoplasm. Terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate-biotin nick end labeling method, × 400.

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degree as the other bromocriptine-treated rats. This leads us to believe that the rat was not completely resistant to bromocriptine, but may have required a larger dose. One of 14 bromocriptine-resistant patients was given increasing doses of bromocriptine over a 9-day period.\textsuperscript{34} While her serum bromocriptine levels rose progressively, serum high PRL levels remained unchanged. The basis of the resistance to dopamine agonist therapy is still unclear.

PCNA is an auxiliary protein of deoxyribonucleic acid (DNA) polymerase-\(\beta\), which is located in the nucleus and is expressed in each phase of the cell cycle except for G\(_0\).\textsuperscript{5,30} Monoclonal anti-PCNA antibody can be used to detect PCNA paraffin-embedded tissue sections.\textsuperscript{14} Cellular proliferation in the anterior pituitary gland in the female rat fluctuates drastically with the highest activity seen during estrus and the lowest during diestrus.\textsuperscript{28} The number of PCNA-positive cells increases after estrogen stimulation.\textsuperscript{6} Bromocriptine suppresses PRL secretion and DNA synthesis in lactotrophic cells via an intracellular negative feedback mechanism.\textsuperscript{5,20} In our study, the PCNA labeling indices of the groups receiving bromocriptine and terguride were approximately one third that of the group receiving no drug. This result suggests that terguride as well as bromocriptine has suppressive effects. Short-term bromocriptine therapy (4 to 6 weeks) causes histological changes including clumping of nuclear chromatin and marked reduction in cytoplasmic volume.\textsuperscript{31,35} No necrosis occurs in connection with these changes.\textsuperscript{35} However, necrotic changes may appear in prolactinomas after long-term bromocriptine administration (14 to 36 weeks).\textsuperscript{36} In our study of short-term bromocriptine and terguride treatment, no necrosis occurred, and cell loss resulted from apoptosis. Therefore, bromocriptine and terguride have cytocidal effects on prolactinoma even after short-term administration.

The TUNEL method is useful for detecting apoptotic cells in situ,\textsuperscript{51} and apoptotic cells in rapidly renewing normal tissues can be located by a modification of this method.\textsuperscript{51} In our study, the TUNEL method detected apoptotic cells in prolactinomas of estrogen-stimulated rats. The number of positively stained cells (apoptotic cell index) in the rats treated with bromocriptine or terguride was nearly double that in the rats given no drug. The apoptotic cell index in estrogen-treated rats given bromocriptine was previously found to be nearly twice that seen in rats receiving no dopaminergic drug.\textsuperscript{12} However, apoptotic cells were counted in sections stained with HE, raising questions of false positives or negatives. Our TUNEL staining method resulted in clear nuclear staining and easy counting of apoptotic cells. The effects of bromocriptine and terguride were similar in suppression of cell proliferation and induction of apoptosis in prolactinomas, but the degree of the effects of terguride was approximately five-fold higher than that for bromocriptine. High numbers of apoptotic cells are observed in medulloblastomas and germinomas, which are considered to be sensitive to radiation and chemotherapeutic agents.\textsuperscript{21} Progressive decreases occurred in serum PRL levels in 26 of 27 females with microprolactinomas treated with both radiotherapy and interim dopamine agonists (bromocriptine, lisuride, pergolide).\textsuperscript{86} These results suggest that the induction of apoptosis by bromocriptine and terguride may have clinical importance as a short-term medication prior to radiotherapy.

References


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Commentary

In the present study, the authors analyzed the effect of bromocriptine and terguride on the estrogen-stimulated anterior pituitary gland of the rat with special attention to the mechanism. The authors found that dopamine agonists such as bromocriptine and terguride induced apoptosis and suppression of the cell proliferation in the prolactin-producing tumor in estrogen-stimulated anterior pituitary gland. They also described that terguride was more effective than bromocriptine. These findings seem to be very important and interesting in respect to considering the treatment of human prolactinomas. However, they did not strictly define which of the suppression of the cell proliferation or induction of the apoptosis was more effective. According to a few apoptosis, the suppression of the cell proliferation may be a main effect. However, PCNA does not necessarily indicate cell proliferation but may relate to the synthesis of peptide hormone. Therefore, further studies including accurate markers for cell proliferation and analysis of the cell cycle are needed to clarify the mechanism.

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The authors present an excellent experimental study, destined to be a significant contribution to the field of pituitary research. They have studied comparatively the cytocidal effects of bromocriptine and terguride in estrogen-prepared rats, showing their suppressive effects on pituitary weight, PRL levels, PCNA levels, number of apoptotic cells, and proliferative cell index, according to the type of drug. It is interesting to note their finding that the degree of the effects of terguride — a partially blocking agent — was five fold higher than that of classical bromocriptine; considering its possible future clinical application as a premedication or “sensitizer” prior to radiotherapy. Specific activity profiles of different dopamine agonists and their different affinity for dopamine — D2 receptors may also elucidate the problem of dopamine resistance that plagues us in daily clinical practice.

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The authors reported the effects of bromocriptine and terguride in the estrogen-induced rat prolactinoma model. Many other studies have reported similar results to those of the present study. Nevertheless, the characteristic of this study may be the analysis of apoptosis in PRL cells treated with each drug. The authors clearly showed the suppression of cell proliferation and induction of apoptosis in the treated group. The TUNEL method, which they used for the detection of apoptotic cells in situ, is so handy and reliable that application to many other fields will be promising.

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