In Vivo Effect of Cabergoline, a Dopamine Agonist, on Estrogen-Induced Rat Pituitary Tumors

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Abstract. Cabergoline (CG) is a dopamine agonist that inhibits the secretion of prolactin (PRL) and growth hormone. In the present study, we evaluated the in vivo effect of CG on PRL secretion and the pituitary tumor induced by estrogen. Estrogen was administered by subcutaneous injection to 4-week-old Fischer 344 rats weekly for 10 weeks to induce tumors. On the last day of estrogen administration, doses of either CG or bromocriptine (BC), 0.6 mg/kg, were administered as a single oral route or chronically, given every third day. Sera and pituitary tumors were sampled on each treatment schedule. Serum levels of PRL were measured and the pituitary glands were weighed. Immunohistological evaluation was performed by optical and electron microscopy. A single dose of CG significantly inhibited the serum levels of PRL for 6 days. Following a single dose of BC, the PRL level was significantly inhibited only at 6 hours' postadministration. The continued oral administration of CG significantly reduced both the serum PRL level and the weight of the pituitary during 15 to 60 days of treatment as compared with BC. Morphologic studies revealed that CG reduced the size of the cells and of the granules, and increased the number of granules per unit area of the cytoplasm. These findings suggest that CG inhibits the maturation of PRL secretory granules and the secretion of PRL more than its synthesis. Thus, CG induced a prolonged lowering of PRL and had a good antitumor effect on rat pituitary tumors induced by estrogen.

Key words: Cabergoline, Bromocriptine, Rat pituitary tumor, Prolactinoma, Prolactin

BROMOCRIPTINE (BC) is known to have a prolactin (PRL)-lowering effect [1] and to reduce the size of rat and human pituitary tumors [2–8]. BC primarily inhibits the release of PRL granules from the cell, which may presumably be related to lowering cyclic AMP levels and increasing intracellular PRL. The former then decreases the rate of PRL synthesis leading to reduction in the size of the cytoplasm and crinophagy related to cell death [9].

Cabergoline (CG), a new oral dopamine agonist, also exhibits a potent PRL-lowering effect and an antitumor effect in animals and humans [10–17]. Its mechanism of action in reducing tumor size has never been reported. We also studied the effect of CG on estrogen-induced rat pituitary tumors in vitro. In that study CG showed a greater action against cultured pituitary tumors than BC [18].

We conducted the present study to examine the changes in serum PRL values and pituitary tumor weight, and analyzed tumor histology after CG treatment by morphologic technique with immunohistochemistry and immunoelectron microscopy, to further investigate the effects of CG in treating pituitary tumors induced in the rat by estrogen.
Materials and Methods

Induction of rat pituitary tumor

Female F344 rats (Charles River Japan Co. Ltd, Yokohama, Japan) 4 weeks old were used. They were housed a room with controlled light and temperature and had free access to laboratory chow and tap water (24 °C and light from 0600 to 2000 h). Pituitary tumors were induced by administering a subcutaneous injection of 2 mg estradiol-17β (E2) in 0.2 ml sesame oil weekly for 10 weeks. No additional estrogen treatment was given thereafter.

Drug administration

To examine the effect of a single dose of each agent on PRL levels, rats were administered either CG orally at a dose of 0.6 mg/kg in 0.2 ml sesame oil, the same dose of BC, or the vehicle sesame oil (0.2 ml), as control. Blood samples were collected from the tail vein under inhalation anesthesia 2 h before administering the single dose and again at 6, 12, 18, and 24 h after the administration, and again at 2, 3, 4, and 6 days.

To examine the effects of the drugs on PRL levels after their chronic oral administration, the rats were administered CG, BC or sesame oil at the doses mentioned by gavage every third day, and were killed by decapitation at 15, 30 and 60 days. The collected sera were stored at -20 °C for radioimmunoassay (RIA) of PRL. The pituitary tissues were removed, weighed, and prepared for examination by light and electron microscopy.

Hormone assays

The PRL standards (NIDDK rat PRL RP-3, AFP-4459B) were provided by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK Bethesda, MD) and the anti rat PRL serum (HAC-RT26-01RBP85) by Dr. Wakabayashi (Institute of Molecular and Cellular Regulation, Gunma University, Maebashi, Japan). Radioiodinated rat PRL was purchased from Du Pont /NEN Research Products (Boston, MA) and goat antirabbit IgG was purchased from Bio Makor (Rehovot, Israel). RIAs of PRL were performed by a double antibody method. In our assay system of rat PRL, the detection range was 156.25 to 4.88 ng/ml.

Light microscopic studies

For light microscopic observations, pituitary tissue was fixed in Bouin’s fluid, dehydrated through a graded series of ethanol, and embedded in paraffin. Sections 5 μm thick were stained with hematoxylin-eosin. To count the number of PRL cells, PRL-secreting cells were identified by the avidin-biotin-peroxidase complex method by using ABC Kit (Vector Lab., Burlingame, CA).

Electron microscopic studies

For ultrastructural observations, pituitary tissue was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for 12 h. Tissue was postfixed in 1% osmium tetroxide for 2 h, dehydrated, and embedded in Spurr’s resin (Taab’s Lab. Equip. Ltd., Berkshire, UK). Ultrathin sections were mounted on copper or nickel mesh.

For immunoelectron microscopic observations, the sections mounted on nickel mesh were etched with 10% H2O2 for 10 min, then rinsed 3 times in phosphate buffer saline (PBS) for 10 min. They were treated with 1% bovine serum albumin in PBS for 40 min and incubated overnight at 4 °C with a dilution of 1:3000 of anti-rat PRL serum (HAC-RT26-01RBP85). After rinsing in PBS, protein-A gold (20-nm particles; EY Lab., Inc., San Mateo, CA) was applied at room temperature for 30 min. They were again rinsed in PBS and distilled water and allowed to dry in air. Sections mounted on copper or nickel mesh were stained with uranyl acetate and lead citrate and observed with an Hitachi H-7000 electron microscope (Tokyo, Japan). For morphometric analysis by immunoelectron microscopy, cellular and nuclear area, number of PRL-immunoreactive granules per μm² cytoplasm, and maximal diameter of these granules were measured from photographic prints.

Statistical method

Data were reported as the mean ± SEM. Differences between two groups were statistically analyzed by Mann-Whitney U test. A level of P<0.05 was accepted as statistically significant.
Results

Serum PRL levels after single or chronic oral dosage

A single oral dose of CG significantly reduced serum PRL levels after 6 h; this inhibitory effect persisted for 6 days (Figs. 1, 2). In contrast, a single oral dose of BC temporarily suppressed serum PRL levels after 6 h, values returned to control (vehicle) levels within 12 h.

The chronic oral administration of CG produced a significant reduction of serum PRL levels for 15 to 60 days. At 60 days after the treatment of CG, serum PRL levels returned to the normal range, approximately 20 ng/ml (Fig. 3). However, no significant reduction in serum PRL levels was observed in the group administered BC.

Pituitary weight

The mean pituitary weight of the rats prior to the chronic administration of the agents was 242.2 mg (Fig. 4). The pituitary weight in the control group gradually increased for 30 days after the final dose of estrogen. CG significantly reduced the pituitary weight on day 15, and it was 29% of the control value on 60 days. In contrast, the weight of the pituitary glands of the BC-treated rats did not differ from that of control. Thus, BC did not
counteract the residual effect of estrogen stimulation on the pituitary tumor.

Light microscopy

Pituitary tumors in the control group appeared as diffuse sheets of similar cells traversed by vascular channels with abundant pools of blood. The tumor tissue treated with CG or BC was partially occupied by acellular spaces and fibrosis, thought to be due to partial necrosis. No such feature was detected in control tumor tissue (Fig. 5).

The PRL tumor cell treated with cabergoline had a shrunken cytoplasm, compared with that of control group (Fig. 6). From the immunohistochemical analysis for PRL, the incidence of PRL cells per about 1,000 cells was 70% in control group, and did not differ significantly among the three groups.

Electron microscopy

Conventional electron microscopic examination verified a marked reduction in the size of individual cells and that of secretory granules in the tumor cells treated with CG as compared with the control (Fig. 7A, B). Degenerative cells in tumor tissue treated with CG were scattered among intact tumor cells (Fig. 7B). From observation by immunoelectron microscopy, most of the tumor cells were found to be immunoreactive with PRL (Fig. 8A, B). Ultrastructural changes in tumor cells containing PRL-immunoreactive granules (PRL tumor cells) were the same as those observed by routine electron microscopy. PRL tumor cells with vacuolized and fragmented rough endoplasmic reticula were also observed (Fig. 9A). In some PRL cells adjacent to the area of fibrosis, condensed and fragmented nuclei and lysis of cytoplasm were also recognized (Fig. 9B). Such lysosomal crinophagic structures were often observed in degenerative cells treated with CG. No degenerative changes were detected in the control group.

The ultrastructural changes observed on immunoelectron microscopy were morphometrically analyzed between control and CG treatment except for the cells showing degenerative features (Table 1, Fig. 10). CG significantly reduced the size of individual cells as compared with control. However, no significant changes in the area of the nucleus were observed between the two groups. Accordingly, the nucleoplasmic ratio was more...
EFFECT OF CABERGOLINE ON RAT PITUITARY TUMORS

Fig. 6.  Immunophotomicrograph of tumor specimen. PRL cell of the control group shows an abundant cytoplasm with localized PRL staining (A). PRL cell treated with cabergoline shows a shrunken cytoplasm with diffuse PRL staining (B). Bar=30 µm.

Fig. 7.  Electronmicrograph of tumor specimen. Ultrathin sections of tumor treated with cabergoline shows a shrunken cytoplasm and cell necrosis (B), compared with that of control group (A). Bar=5 µm.

Fig. 8.  Immunoelectronmicrograph of tumor specimen. Tumor cell treated with cabergoline shows a shrunken cytoplasm and reduction in size of secretory granules (B), compared with tumor cells of the control group (A). Bar=2 µm.
marked in the CG group than in the control group. In addition, CG significantly reduced the maximal diameter of the PRL-immunoreactive secretory granules and increased the number of granules per unit cytoplasmic area (Table 1). Analysis of diameter distribution revealed that secretory granules with a smaller diameter occupied a greater part of the population in the CG-treated group than in the control group (Fig. 10).

### Discussion

Estrogen directly acts on the rat anterior pituitary gland and induces the proliferation and hypertrophy of lactotrophs. Chronic administration of estrogen to rats ultimately produces a PRL-secreting pituitary tumor with dopamine receptors similar to those of a normal pituitary [8, 19–21]. PRL secretion is regulated via dopamine and GAP (gonadotropin releasing hormone associated peptide). The nature of the intracellular messenger has not been completely elucidated [22]. BC binds with the D2 receptor and inhibits PRL exocytosis via an inhibition of adenylate cyclase activity and a reduction in the level of cyclic AMP (adenosine monophosphate) [9, 23], leading to the intracellular accumulation of PRL. As a result, the synthesis of PRL and of DNA are decreased, leading to a reduction in mitotic activity. Calcium channels and inositol triphosphate signaling seem to be involved in this signal transduction. A deg-

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<th>Table 1. EM morphometric characteristics of PRL cells in rat pituitary tumor obtained at 60 days after administration of cabergoline*</th>
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*Values are means ± SEM. These values were obtained from 37 cells (control) and 42 cells (CG) randomly selected in 4 rats. Differences from control, $P<0.05$.  

Fig. 9. Immunelectronmicrograph showing various stages of cell necrosis. Bar=2 $\mu$m. Vacuolized and fragmented rough endoplasmic reticula are observed in PRL cell (arrows, A). Condensed and fragmented nucleus (Nu) and lysosome (arrow head) in the cytoplasm are also observed in the PRL cell adjacent to the fibrosis (B).

Fig. 10. Repartition curve of diameter of PRL-granules. Curve of cabergoline rises in the lower part of the graph. Difference between two groups, $P<0.001$.  

EGUCHI et al.
radation of PRL accumulated in lactotrophs may be also involved in the inhibitory effect of BC on PRL secretion [24].

CG is a new oral dopaminergic agent that shows a potent PRL-lowering effect in rats and humans [11-14, 17, 25]. It inhibits the tumorigenesis of the rat pituitary gland stimulated by estrogen and the enlargement of the estrogen-induced rat pituitary tumor [10, 16].

The inhibitory effect following a single oral dose of CG was more potent than that of BC, persisting for 6 days, in general agreement with other findings [10, 11]. Those authors found that a single oral dose of CG induced a marked fall in serum PRL level that persisted for 72 h in rats [10], and for 7 days in humans [15].

The disappearance half-life of CG is biphasic like that of other ergoline derivatives, being 7.5 (T 1/2 = 24 h) and 65 h (T 1/2 = 120 h). The corresponding values for BC are 6.5 and 67.9 h, respectively. Thus, CG and BC have similar half-lives. The bioavailability of CG by the oral route ranges from 7.4–17.8% in humans, while that of BC is about 6% [26]. The affinity of CG for binding to dopamine receptors in the strial region of rat brain exceeds that of BC [27]. The prolonged effect of CG on serum PRL levels may be due to its improved bioavailability and a higher affinity for dopamine receptors as compared with BC. Chronic oral administration of CG also induced a marked decrease in serum PRL levels, with values returning to the normal range 60 days after treatment. A long-lasting dopamine agonist such as CG may be useful in treating patients with hyperprolactinemia who are intolerant of BC.

Many studies have demonstrated a reduction in the size of human pituitary tumors during long-term treatment of BC [3, 4, 9, 28-30]. The efficacy of BC on estrogen-induced rat pituitary tumors has also been reported. These studies revealed that BC achieves the following: inhibition of tumor growth [31, 32], reduction in the size of tumor cells [4, 7, 33, 34], appearance of degenerative or necrotic features [3, 4], nucleolar change i.e. pycnosis, karyorrhexis and karyolysis [3], and reduction in the size of organelles involved in PRL synthesis [5, 7].

In our comparison of CG and BC, we used the same oral dose of each agent. At that dose CG, but not BC, was effective in reducing the weight of pituitary tumor. This indicates that a higher dose of BC is required to obtain an antitumor effect. This seems to be supported by the finding that the administration of CG every third day was as effective as daily BC administered at a five-fold higher dose than CG in suppressing the development of estrogen-induced pituitary tumor [10]. The present study also demonstrated that CG reduced the size of tumor cells, produced tumor cell necrosis, and led to the disappearance of pools of blood in tumor tissue. A decrease in tumor weight may also have resulted. Such effects of CG agree with those observed with BC therapy [7], which suggests a similar action of CG and BC.

Morphologic studies revealed that CG reduced the size of PRL-immunoreactive secretory granules and increased the number of granules per unit area of cytoplasm. These findings accorded well with those of BC in previous studies and of castrated rats, which may due to the decreased activity of Golgi apparatus followed by change in the processing and maturation of secretory granules [5, 8, 34-36]. The present results therefore suggest that CG preferentially inhibits the maturation of the PRL secretory granule.

In conclusion, the antitumor effect of CG resembled that of BC. CG exhibited superior features including a prolonged-lowering of PRL, a good antitumor effect against estrogen-induced rat pituitary tumors, and can be used at a lower dose and frequency of administration.

Acknowledgments

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