Induction of apoptosis in murine ACTH-secreting pituitary adenoma cells by bromocriptine

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Abstract

Bromocriptine, a dopamine agonist, is now an accepted primary therapeutic agent for patients with prolactinomas and other pituitary adenomas. In this study, we demonstrated that bromocriptine inhibited the proliferation of murine ACTH-secreting pituitary adenoma (AtT-20) cells. In addition, the antitumor activity of bromocriptine was inhibited both by actinomycin D and cycloheximide, suggesting that it was dependent on new RNA and protein synthesis. Interestingly, the results of DNA fragmentation assays and cell cycle analysis clearly demonstrated that bromocriptine induced apoptosis in AtT-20 cells.

Key words: Apoptosis; Pituitary tumor; Bromocriptine

1. Introduction

Apoptosis (programmed cell death) [1] can be described as a process in which cells actively participate in their own death. The term apoptosis has been used to describe the death of cells killed during normal development or by growth factor deprivation [2-4]. Apoptotic dying cells exhibit characteristic changes, including chromatin condensation, membrane blebbing, and fragmentation of the DNA into nucleosome-sized pieces. The DNA fragmentation which occurs yields a ladder pattern in agarose gel and is a hallmark of apoptotic cell death. Recently, apoptosis has also been the focus of research since it can result from the administration of antitumor chemotherapeutic drugs such as camptothecin [5], cisplatin [6] and etoposide [7].

Bromocriptine, a dopamine agonist, is now an accepted therapeutic agent for patients with prolactinomas, GH-secreting pituitary adenomas [8-12], and ACTH-secreting pituitary adenomas [13]. Bromocriptine decreases elevated serum level of prolactin or growth hormone and shrinks such tumors by diminishing tumor cell size. Ultrastructural studies following bromocriptine administration demonstrate nuclear pyknosis, aggregated chromatin, reduced volume densities of rough endoplasmic reticulum and Golgi apparatus, a significant reduction in the volume of the cytoplasm, and atrophy or swelling of mitochondria [14-16]. The above findings and those of other biochemical studies together make clear that bromocriptine inhibits hormone and DNA biosynthesis [17,18] and has antimitotic effects [19,20]. However, the mechanisms by which bromocriptine inhibits growth are not yet known in detail. In this study, therefore, we used a DNA fragmentation assay and cell cycle analysis in attempting to determine whether bromocriptine induces apoptosis in murine ACTH-secreting pituitary adenoma (AtT-20) cells.

2. Materials and methods

2.1. Materials

2.1.1. Chemotherapeutic agent. Bromocriptine was the generous gift of Sandoz Pharmaceutical Co., Ltd. (Tokyo, Japan). It was obtained in powder form, from which a 10 mM stock solution was prepared in methanol.

2.1.2. Cells. Murine ACTH-secreting pituitary tumor (AtT-20) cells were kindly provided by the Japanese Cancer Research Resources Bank (Tokyo, Japan). AtT-20 cells were maintained in suspension culture in Ham's F10 containing glutamine, 15% horse serum, 2.5% fetal calf serum, and the antibiotics penicillin and streptomycin.

2.2. Methods

2.2.1. Cell viability. The cytotoxic effects of bromocriptine on AtT-20 cells were quantified using a modified MTT (CHEMICON, Temecula, CA) calorimetric assay [21]. Cells were seeded at 10^4 cells/ well (0.1 ml) in 96-well flat-bottomed plates (Corning, NY) and treated with varying doses of bromocriptine between 0.1 µg/ml and 40 µg/ml. Following a 72-h period of incubation at 37°C, 0.01 ml of MTT reagent was added to each well. Following another 4-h period of incubation at 37°C, 0.1 ml of isopropanol with 0.04 N HCl was added to each well to dissolve precipitates, and absorbance was then measured at 570 nm with an autoreader (ER-8000, Sanko Junyaku Co., Ltd., Tokyo, Japan) within 30 min of dissolution.

2.2.2. Inhibition of RNA and protein synthesis. To determine whether inhibition of RNA or protein synthesis results in inhibition of the

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Abbreviations: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PBS, phosphate-buffered saline.
cycloheximide (0.4 µg/ml) to inhibit protein synthesis, prior to chemotherapy. Changes induced in cytotoxicity were then quantified using a modified MTT assay.

2.2.3. Analysis of DNA fragmentation in agarose gels. This assay was performed using methods previously described [22]. Briefly, harvested cells (1 x 10⁶) were centrifuged and washed twice with cold PBS. The cell pellet was lysed in 1.0 ml of a buffer consisting of 10 mM Tris-HCl, 10 mM EDTA and 0.2% Triton X-100 (pH 7.5). After 10 min on ice, the lysate was centrifuged (13,000 x g) for 10 min at 4°C in an Eppendorf microfuge. Then, the supernatant (containing RNA and fragmented DNA, but not intact chromatin) was extracted first with phenol and then with phenol-chloroform/isomyl alcohol (24:1). The aqueous phase was brought to 300 mM NaCl and nucleic acids were precipitated with 2 vol of ethanol. The pellet was rinsed with 70% ethanol, air-dried and then dissolved in 20 µl of 10 mM Tris-HCl, 1 mM EDTA (pH 7.5). Following digestion of RNA with RNase A (0.6 mg/ml, at 37°C for 30 min), the sample was electrophoresed in a 2% agarose gel with Boyer’s buffer (50 mM Tris-HCl, 20 mM sodium acetate, 2 mM EDTA and 0.2% Triton X-100 (pH 7.5). After 10 min on ice, the cell’s DNA into nucleosome-sized units [24,25]. In this study, we also attempted to determine whether bromocriptine induces apoptosis in AtT-20 cells. Our findings show that DNA fragmentation was clearly induced in AtT-20 cells after treatment for 72 h with bromocriptine (Fig. 2).

3. Results and discussion

Pituitary adenomas, particularly prolactinomas, are not rare. The reported incidence of microadenomas varies between 23% and 27% [23]. The goals of therapy for pituitary adenomas always include reduction of tumor mass, restoration of normal visual field and cranial nerve function, preservation of other anterior pituitary functions, suppression of excessive hormone secretion, and prevention of recurrence or progression of the disease. Bromocriptine has been used not only for treatment of mild hyperprolactinemia and microadenomas but also as initial therapy for large prolactinomas and persistent postoperative hyperprolactinemia. There have been many reports of its efficacy in lowering serum prolactin level, reducing tumor size, improving visual field abnormalities, and restoring gonadal function. However, it is not yet clear how bromocriptine exerts its antitumor effects on pituitary adenomas. As shown in Fig. 1, bromocriptine inhibited the proliferation of AtT-20 cells in a dose-dependent fashion. In addition, the decrease in AtT-20 cell viability induced by bromocriptine is almost entirely dependent on RNA and protein synthesis, since actinomycin D and cycloheximide each prevent cell death in part. These findings demonstrate that bromocriptine can kill pituitary tumor cells in vitro, and that RNA and protein synthesis are required for the induction of cell death by bromocriptine.

Apoptosis is characterized by cell shrinkage, and also by DNA fragmentation due to the activation of an uncharacterized Ca²⁺/Mg²⁺ endonuclease which cleaves the cell’s DNA into nucleosome-sized units [24,25]. In this study, we also attempted to determine whether bromocriptine induces apoptosis in AtT-20 cells. Our findings show that DNA fragmentation was clearly induced in AtT-20 cells after treatment for 72 h with bromocriptine (Fig. 2).

We also studied the changes in the intensity of fluorescence of DNA using flow cytometry. As shown in Fig. 3, bromocriptine treatment of AtT-20 cells resulted in a decrease in the percentage of cells in G0/G1 phase and an increase in percentage of cells in S and G2/M phases,
In conclusion, our findings suggest that the dopamine agonist bromocriptine has a cytotoxic effect on and induces apoptosis of pituitary adenoma cells.

We are at present attempting to determine whether bromocriptine induces accumulation of the tumor-suppressor protein p53 in pituitary adenoma cells when it initiates apoptosis.

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References