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The effect of octreotide and bromocriptine on expression of a pro-apoptotic Bax protein in rat *prolactinoma*

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Abstract: It is well established that disruption of apoptosis may lead to tumor initiation, progression or metastasis. It is also well documented that many anticancer drugs induce apoptosis. In the earlier studies, the dopamine D₂ receptor agonist bromocriptine (BC) and somatostatin analog octreotide (OCT) were found to inhibit the growth of the estrogen-induced rat *prolactinoma*. Our previous investigations, applying the TUNEL method showed the involvement of the pro-apoptotic effect in the action of BC, and to a lesser degree, in the action of OCT. The aim of the present study was to investigate whether the pro-apoptotic action of these drugs involves the increased expression of Bax - a member of Bcl-2 protein family which is known to play an important role in the regulation of apoptosis. Male four-week Fisher 344 rats were used in the experiment. Capsules containing diethylstilboestrol (DES) were implanted subcutaneously. Six weeks after the implantation the rats were given OCT (2 × 25 µg/animal/24), BC (3 mg/kg b.w./24 h) or OCT and BC at the above doses for 10 days. Bax expression was detected by immunohistochemistry. Prolactin (PRL) in blood serum was measured by radioimmunoassay (RIA). It has been found that both OCT and BC, alone or in combination, significantly reduce the tumor weight. Both OCT and BC suppressed PRL levels, but the inhibitory effect of BC was stronger than that of OCT. It has been found that the treatment with OCT and BC, alone or in combination, causes a significant increase in Bax expression in the rat *prolactinoma* cells. Our findings indicate that anti-tumoral action of bromocriptine and to some extent the action of octreotide in the experimental rat *prolactinoma* is connected with the induction of apoptosis and is associated with increased Bax expression.

Key words: Octreotide - Bromocriptine - Bax - Apoptosis - *Prolactinoma*, experimental

Introduction

The cell death may be the result of either necrosis or apoptosis. In contrast to necrosis, apoptosis is a complex, gene-directed process whereby individual cells are triggered to undergo self-destruction. The major biochemical event considered as a hallmark of apoptosis is the cleavage of DNA into oligonucleosomal (180-200-bp) fragments (for review see [12, 21, 23]).

The Bcl-2 (B cell leukemia/lymphoma-2) family of proteins plays an important role in the regulation of apoptosis (for review see [1, 8, 20]). It includes both the death-promoting as well as the death-inhibiting members. One of the death-promoting proteins is Bax.

The studies concerning apoptosis in the pituitary gland show that the apoptotic index in both nontumorous

and adenomatous pituitary is low [7, 14, 17]. Bromocriptine - D₂ dopamine receptor agonist, has been shown to induce apoptosis in pituitary tumors *in vivo* [5, 36] and *in vitro* [11, 31, 33, 34, 35]. However, other studies provide contradictory results [13, 14, 25]. The data concerning apoptosis in pituitary tumors after somatostatin analog treatment are also controversial. The induction of apoptosis has been reported only in a few studies *in vivo* [30] and *in vitro* [24]. However, the other studies have not revealed apoptosis in pituitary tumors after somatostatin analog therapy either *in vivo* [18, 22] or *in vitro* [4].

In our previous paper, we examined the effects of D₂ dopamine receptor agonist bromocriptine and somatostatin analog octreotide on apoptosis (visualized by the TUNEL method) in the rat prolactin-secreting pituitary tumor [9]. We found that both bromocriptine and octreotide enhanced the number of apoptotic cells in the tumor, but the pro-apoptotic effect of bromocriptine was more pronounced. Unexpectedly, the joint effect of octreotide and bromocriptine was much lower than that of bromocriptine alone.

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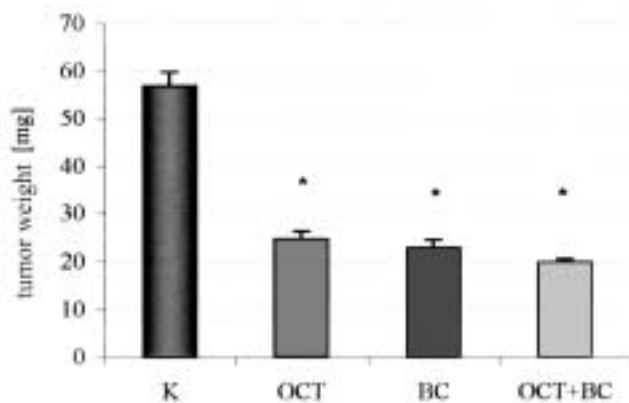


Fig. 1. Effects of octreotide (OCT) and bromocriptine (BC) on tumor weight in rat estrogen-induced *prolactinoma*. * $p < 0.05$ vs K (control).

In the present study we investigate whether the proapoptotic action of these drugs involves the increased expression of the proapoptotic Bax protein.

Materials and methods

Four week old male Fischer 344 rats weighing 50-70 g, maintained in controlled illumination regime (12L/12D), with free access to standard laboratory food and tap water, were used in the experiment. Capsules containing 8-10 mg of diethylstilboestrol (DES, Sigma) each were implanted subcutaneously (s.c.) in the lumbar region. Such capsules were estimated to release 18-45 μg of DES daily [32].

Six weeks after the implantation of capsules the rats were divided into 5 groups and treated with the following substances for 10 days: Group IA - control: 0.25 ml of physiological saline s.c., once daily; Group IB - control: 0.25 ml of 50% ethanol in physiological saline s.c., twice daily; Group II: octreotide (OCT, Sandostatin, Novartis) at a dose of 25 μg /animal s.c., twice daily; Group III: bromocriptine (BC, Bromocriptine mesylate, Lek) at a dose of 3 mg/kg b.w. s.c., once daily; Group IV: OCT + BC at the above doses. BC was dissolved in 50% ethanol in physiological saline.

On the eleventh day the animals were sacrificed. Blood and pituitaries were collected. The glands were weighed, fixed in 4% formalin in phosphate buffered saline and then embedded in paraffin wax. The experimental protocol was approved by the Local Ethical Committee for Animal Experimentation (decision Nr Ł/BD/61, 11 June 2001).

Bax expression was detected by immunohistochemistry using commercial monoclonal antibodies (Polyclonal rabbit anti-mouse/rat Bax antibody, Pharmingen, Becton Dickinson Company, San Diego, CA 92121, USA) and DAKO EnVisionTM+ System, HRP/DAB (rabbit ready-to-use detection system using the labelled polymer method, DAKO Corporation, Carpimena, CA 93013, USA) - a kit containing three bottles. The paraffin sections were dewaxed and subjected to microwave antigen retrieval in 10 mM citrate buffer (pH 6.0) for 5 min twice. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 15 min. The sections were then preblocked with 5% normal sheep serum for 30 min and incubated with anti-Bax antibody (1:2000) for 2 h at a room temperature. Sections were then washed and incubated with secondary antibody for 30 min. The reaction product was visualized by diaminobenzidine tetrachloride after 4 min of incubation. Finally, the sections were counterstained with haematoxylin. Negative control was performed by omitting anti-Bax antibodies. Mice thymus was used as a positive control.

Bax expression was evaluated by counting at random 3000 cells from each section at $\times 600$ magnification. The number of Bax-im-

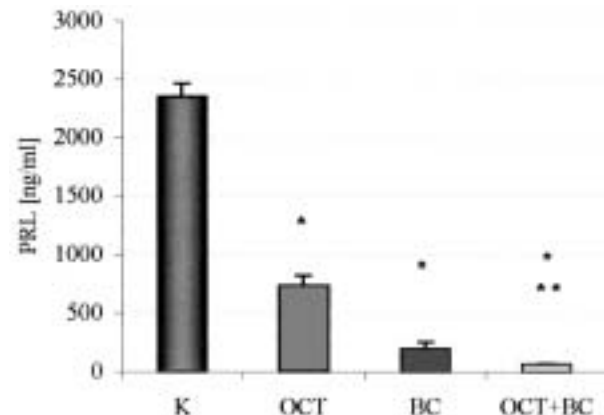


Fig. 2. Effects of octreotide (OCT) and bromocriptine (BC) on serum prolactin (PRL) level in rat estrogen-induced *prolactinoma*. * $p < 0.05$ vs K (control), ** $p < 0.05$ vs OCT.

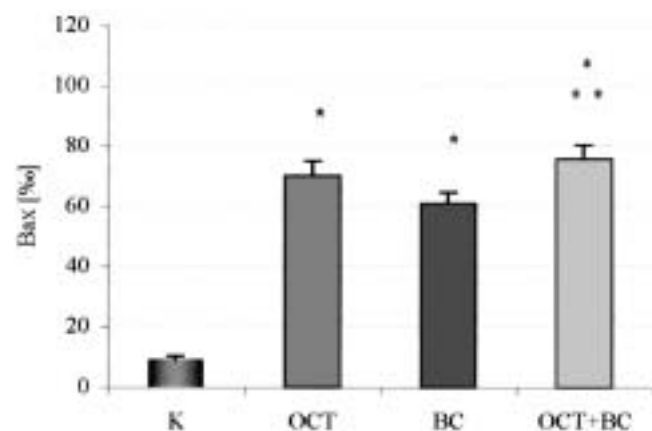


Fig. 3. Effects of octreotide (OCT) and bromocriptine (BC) on Bax expression in rat estrogen-induced *prolactinoma* cells. * $p < 0.05$ vs K (control), ** $p < 0.05$ vs BC.

munopositive cells per 1000 was assessed in each section. Only the cells exhibiting a strong, specific cytoplasmic staining were counted. The 0.5 mm boundary around the section was excluded from analysis to circumvent artifactual staining which may occur at the edges of tissue sections.

Prolactin (PRL) was assayed in blood serum using the rat prolactin ¹²⁵I assay system with magnetic separation (Amersham, UK) and expressed in ng/ml. The method sensitivity is ~ 0.7 ng/tube (7.0 ng/ml).

Statistical analysis was performed using ANOVA followed by LSD test. $P < 0.05$ was considered as the borderline of statistic significance. As there was no statistically significant difference between both control groups, they were pooled.

Results

Six weeks after the implantation of DES, the animals of the control group exhibited the tumorous enlargement of the anterior pituitary. The data concerning the tumor weight are shown in Figure 1. It was found that both OCT and BC, alone or in combination, significantly reduced the tumor weight.

The animals implanted with DES had very high PRL levels. All treatment options resulted in a statistically

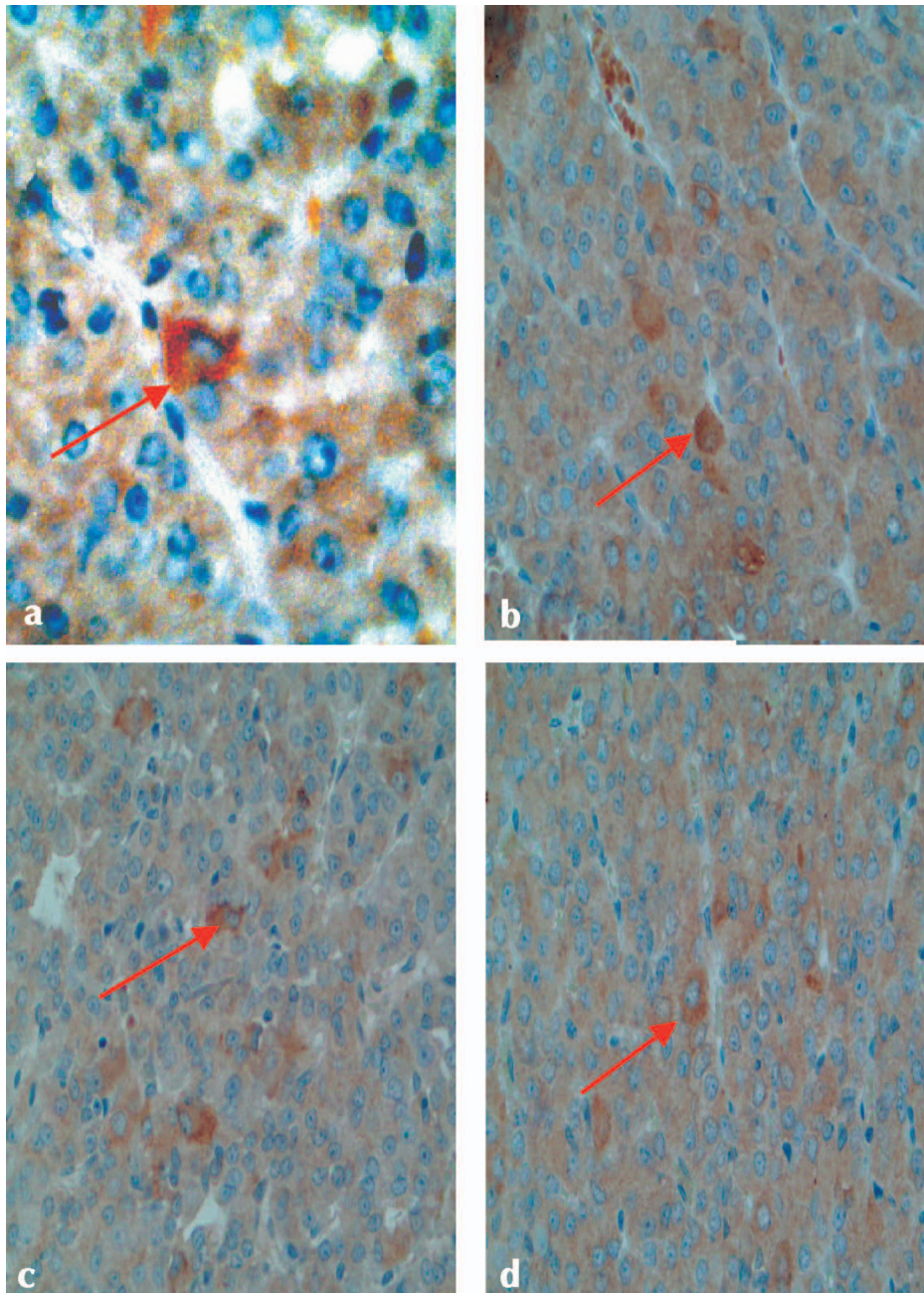


Fig. 4. Immunostaining for Bax in rat estrogen-induced *prolactinoma* in untreated rats (a) and after octreotide (b), bromocriptine (c) and combined treatment with both drugs (d). A: × 400, B-D: × 200.

significant reduction of PRL serum level (Fig. 2). The effect of BC was stronger than that of OCT. The joint action of OCT and BC caused a more pronounced decrease of PRL level as compared to OCT alone.

The treatment with both OCT and BC, alone or in combination, caused a significant (approx. tenfold) increase in Bax expression in the rat *prolactinoma* cells. The joint effect of OCT and BC was stronger than that of BC alone (Figs. 3, 4).

Discussion

In the only previous study concerning Bax expression in pituitary tumors, Bax was demonstrated using immu-

nohistochemistry to be moderately expressed in normal human pituitary and in all examined human pituitary adenomas, from patients with or without a preoperative treatment either with octreotide or with dopamine agonists [14]. Bax expression was decreased in pituitary carcinomas. Bcl-X and Bad, two other pro-apoptotic proteins and Bcl-2, exhibiting anti-apoptotic properties, were expressed at a similar level to Bax. Bcl-2 was expressed in ~70% of pituitary adenomas.

Bcl-2 expression in human pituitary tumors was also investigated by Wang *et al.* [29], who detected Bcl-2 immunoreactivity in 30% of tumors and in none of normal pituitaries. Turner *et al.* [27] examined 160 human pituitary adenomas and found Bcl-2 expression

in over 50% tumors including over 70% *prolactinomas*. Higher Bcl-2 expression was found in the more vascular tumors. Unfortunately, in both studies cited above no data concerning previous pharmacotherapy of the pituitary tumors are shown.

In vitro studies concerning two pituitary adenoma cell lines (GH₃ and AtT-20) revealed a decreased expression of Bcl-2 and an accumulation of the wild-type p53 associated with bromocriptine-induced apoptosis [35]. It has been also found that the cell remodeling of the anterior pituitary after the termination of lactation occurs through the process of apoptosis and involves an increase in Bax and a decrease in Bcl-2 expression [2].

In the present study we demonstrate an increased expression of Bax in a rat prolactin-secreting pituitary tumor after bromocriptine and octreotide treatment, as compared with the untreated tumors. In the light of earlier immunohistochemical investigations [15] which revealed that DES-induced rat pituitary tumors contained almost solely lactotrophs, it can be assumed that the increased Bax expression concerns lactotrophs. In our previous study [9] concerning the same experimental model we demonstrated using the TUNEL method that the antitumoral action of bromocriptine and to some extent the action of octreotide, was connected with the induction of apoptosis. The joint administration of octreotide and bromocriptine resulted unexpectedly in the attenuation of the pro-apoptotic effects of the latter. The attenuation of the pro-apoptotic action of bromocriptine by octreotide may be the result of the cytoprotective effect of somatostatin analogs. Cytoprotection is an organ-specific reduction or prevention of vulnerability of cells and tissues against injury [28]. Cytoprotective properties of somatostatin and its analogs have been demonstrated in several experimental models, especially in the gastrointestinal tract [16, 19, 26]. It has also been found that cytoprotective effects of somatostatin can be dissociated from its endocrine effects, suggesting that different mechanisms are involved in cytoprotection and secretory cell inhibition [28]. Cytoprotective action of somatostatin and its analogs has not yet been investigated in the pituitary. The hypothesis presented above needs to be verified.

In the present study, similar intensity of Bax immunostaining was observed after bromocriptine and octreotide, administered separately or in combination. A question arises how this discrepancy could be explained. It should be stressed that the TUNEL method is based on the detection of oligonucleosomal DNA fragments which are characteristic of cells in the later stage of the apoptotic process [6]. It is possible that high susceptibility to apoptosis evidenced by Bax expression does not necessarily commit all Bax-immunopositive cells to the apoptotic cell death.

In conclusion, our results suggest that anti-tumoral action of bromocriptine and to some extent the action of

octreotide in the experimental rat *prolactinoma* is connected with the induction of apoptosis and associated with increased Bax expression.

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