FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 42, No. 1, 2004
pp. 35-39

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

Anna Gruszka[1], Jolanta Kunert-Radek[2] and Marek Pawlikowski[1]

[1]Department of Experimental Endocrinology and Hormone Diagnostics;
[2]Department of Clinical Endocrinology, Institute of Endocrinology, Medical University, Łódź, Poland

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 D_2 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 - D_2 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 D_2 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.

Correspondence: A. Gruszka, Institute of Endocrinology, Medical University, Sterlinga 3, 91-425 Łódź, Poland;
e-mail: agruszka@mp.pl

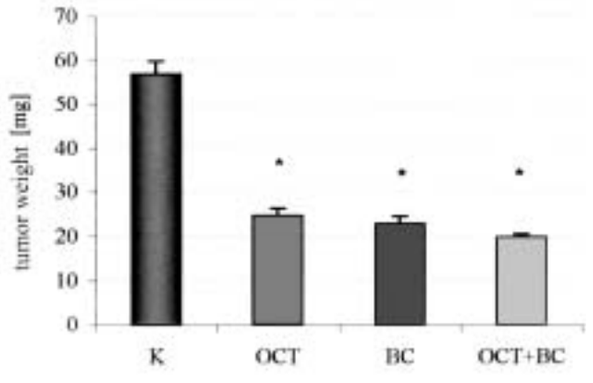

Fig. 1. Effects of octreotide (OCT) and bromocriptine (BC) on tumor weight in rat estrogen-induced *prolactinoma*. *p<0.05 vs K (control).

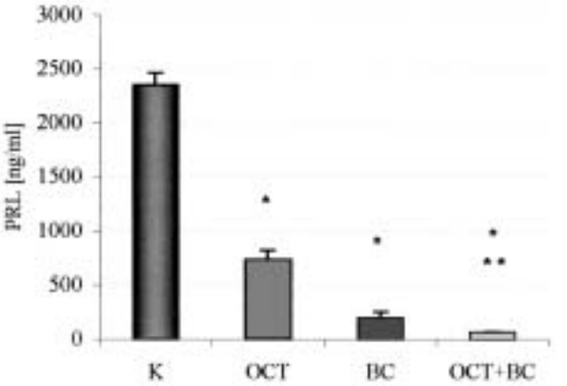

Fig. 2. Effects of octreotide (OCT) and bromocriptine (BC) on serum prolactin (PRL) level in rat estrogen-induced *prolactinoma*. *p<0.05 vs K (control), **p<0.05 vs OCT.

In the present study we investigate whether the pro-apoptotic action of these drugs involves the increased expression of the pro-apoptotic 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 EnVision^{TM}+ 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 tetrachydrochloride 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 × 600 magnification. The number of Bax-immunopositive 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.

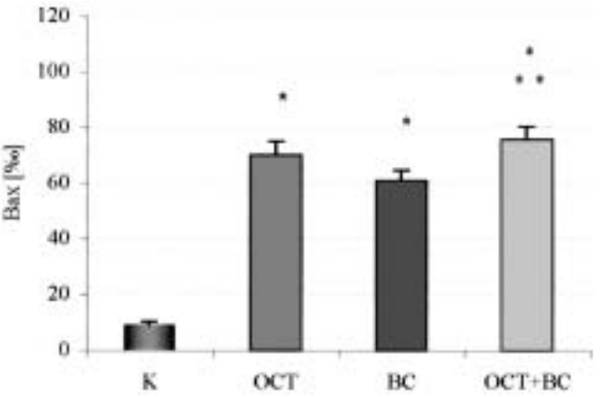

Fig. 3. Effects of octreotide (OCT) and bromocriptine (BC) on Bax expression in rat estrogen-induced *prolactinoma* cells. *p<0.05 vs K (control), **p<0.05 vs BC.

Prolactin (PRL) was assayed in blood serum using the rat prolactin ^{125}I 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

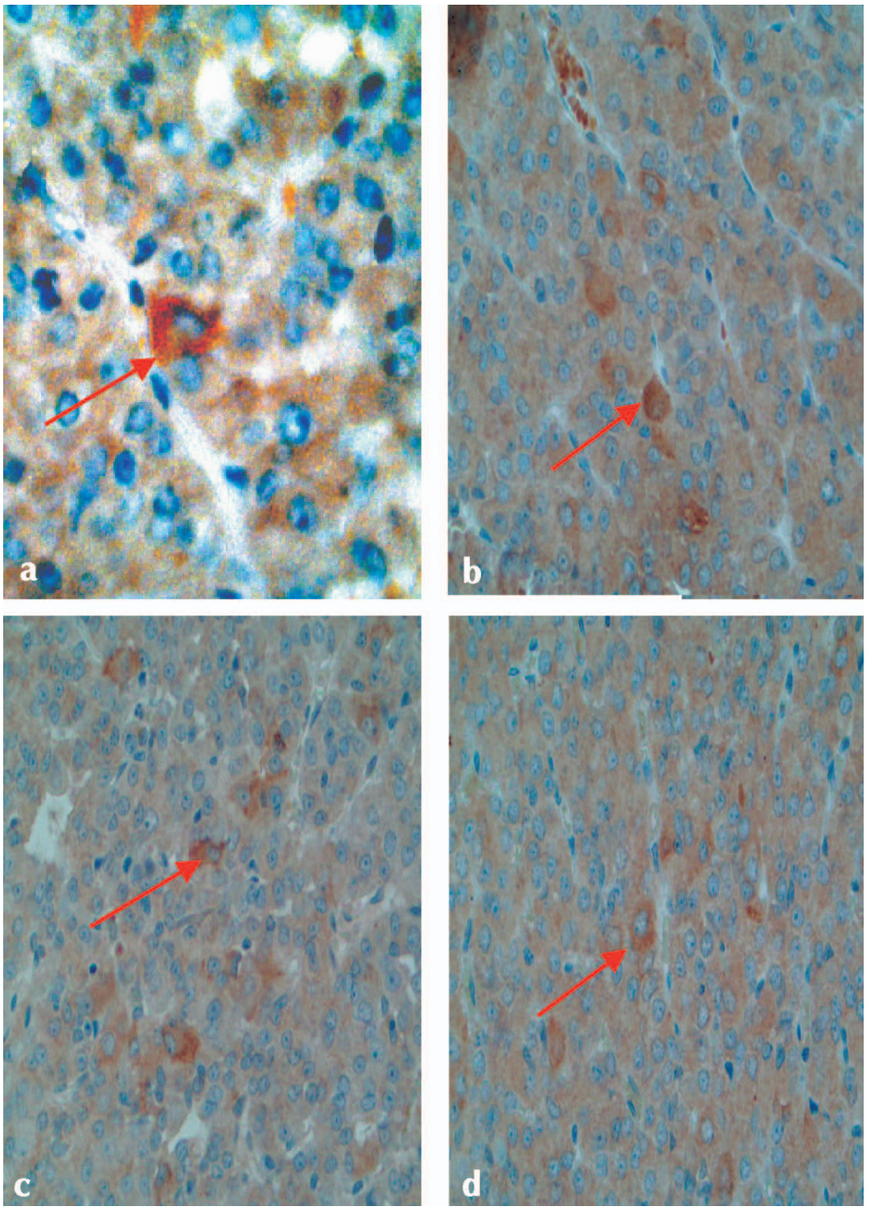

Fig. 4. Immunostaining for Bax in rat estrogen-induced *prolactinoma* in untreated rats (**a**) and after octreotide (**b**), bromocriptine (**c**) and combined treatment with both drugs (**d**). A: × 400, B-D: × 200.

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 immunohistochemistry 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 (GH_3 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.

Acknowledgements: This paper was supported by the Medical University of Łódź. The authors thank Dr. Katarzyna Winczyk for her valuable suggestions concerning Bax immunohistochemistry and Mrs Maria Jaranowska, Mrs Małgorzata Jędrzejewska and Mrs Anna Opłatowska for their skillful technical assistance.

References

[1] Adams JM, Cory S (1998) The Bcl-2 protein family: arbiters of cell survival. Science 28: 1322-1326
[2] Ahlbom E, Grandison L, Zhivotovsky B, Ceccatelli S (1998) Termination of lactation induces apoptosis and alters the expression of the Bcl-2 family members in the rat anterior pituitary. Endocrinology 139: 2465-2471
[3] Bouillet P, Strasser A (2002) BH3-only proteins - evolutionarily conserved pro-apoptotic Bcl-2 family members essential for initiating programmed cell death. J Cell Sci 115: 1567-1574
[4] Cheung NW, Boyages SC (1995) Somatostatin-14 and its analog octreotide exert a cytostatic effect on GH_3 rat pituitary tumor cell proliferation via a transient G0/G1 cell cycle block. Endocrinology 136: 4174-4181
[5] Drewett N, Jacobi JM, Willgoss DA, Lloyd HM (1993) Apoptosis in the anterior pituitary gland of the rat: studies with estrogen and bromocriptine. Neuroendocrinology 57: 89-95
[6] Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death *in situ via* specific labeling of nuclear DNA fragmentation. J Cell Biol 119:493-501
[7] Green VL, White MC, Hipkin LJ, Jeffreys RV, Foy PM, Atkin SL (1997) Apoptosis and p53 suppressor gene protein expression in human anterior pituitary adenoma. Eur J Endocrinol 136: 382-387
[8] Gross A, McDonnell JM, Korsmeyer SJ (1999) Bcl-2 family members and the mitochondria in apoptosis. Genes Dev 13: 1899-1911
[9] Gruszka A, Pawlikowski M, Kunert-Radek J (2001) Anti-tumoral action of octreotide and bromocriptine on the experimental rat prolactinoma: anti-proliferative and pro-apoptotic effects. Neuroendocrinol Lett 22: 343-348
[10] Kamijo K, Sato M, Saito T, Yachi A, Minase T (1991) Effects of bromocriptine on experimental GH_3 cell tumors. Pathol Res Pract 187: 593-597
[11] Kanasaki H, Fukunaga K, Takahashi K, Miyazaki K, Miyamoto E (2000) Involvement of p38 mitogen-activated protein kinase activation in bromocriptine-induced apoptosis in rat pituitary GH_3 cells. Biol Reprod 62: 1486-1494
[12] Kawiak J, Hoser G, Skórski T (1998) Apoptosis and some of its medical implications. Folia Histochem Cytobiol 36: 99-110
[13] Kontogeorgos G, Sambaziotis D, Piaditis G, Karameris A (1997) Apoptosis in human pituitary adenomas: a morphologic and *in situ* end-labeling study. Mod Pathol 10: 921-926
[14] Kulig E, Jin L, Qian X, Horvath E, Kovacs K, Stefaneanu L, Scheithauer BW, Lloyd RV (1999) Apoptosis in nontumorous and neoplastic human pituitaries. Expression of the Bcl-2 family of proteins. Am J Pathol 154: 767-774
[15] Kunert-Radek J (1989) The effect of benzodiazepines and calcium channel modulators on the proliferation of rat prolactinoma - *in vitro* studies (in Polish). Thesis, AM Łódź
[16] Limberg B, Kommerell B (1983) Beneficial effect of somatostatin on galactosamine induced liver injury. Regul Pept 7: 187-193
[17] Losa M, Barzaghi RLA, Mortini P, Franzin A, Mangili F, Terreni MR, Giovanelli M (2000) Determination of the prolife-

ration and apoptotic index in adrenocorticotropin-secreting pituitary tumors. Comparison between micro- and macroadenomas. Am J Pathol 156: 245-251
[18] Losa M, Ciccarelli E, Mortini P, Barzaghi R, Gaia D, Giuliano Faccani G, Mauro Papotti M, Mangili F, Terreni MR, Camanni F, Giovanelli M (2001) Effects of octreotide treatment on the proliferation and apoptotic index of GH-secreting pituitary adenomas. J Clin Endocrinol Metab 86: 5194-5200
[19] Rao GS, Lemoch H, Kessler H, Damm I, Eiermann V, Koll S, Zarbock J, Usadel KH (1986) Prevention of phalloidin-induced lesions on isolated rat hepatocytes by novel synthetic analogues of somatostatin. Klin Wochenschr 64, Suppl 7: 79-86
[20] Reed JC (1998) Bcl-2 family proteins. Oncogene 17: 3225-3236
[21] Reed JC (2000) Mechanisms of apoptosis. Am J Pathol 157: 1415-1430
[22] Saitoh Y, Arita N, Ohnishi T, Ekramullah S, Takemura K, Hayakawa T (1997) Absence of apoptosis in somatropinomas treated with octreotide. Acta Neurochir (Wien) 139: 851-856
[23] Soini Y, Pääkö P, Lehto V-P (1998) Histopathological evaluation of apoptosis in cancer. Am J Pathol 153: 1041-1053
[24] Srikant CB (1995) Cell cycle dependent induction of apoptosis by somatostatin analog SMS 201-995 in AtT-20 mouse pituitary cells. Biochem Biophys Res Commun 209: 400-406
[25] Stefaneanu L, Kovacs K, Scheithauer BW, Kontogeorgos G, Riehle DL, Sebo TJ, Murray D, Vidal S, Tran A, Buchfelder M, Fahlbusch R (2000) Effect of dopamine agonists on lactotroph adenomas of the human pituitary. Endocr Pathol 11: 341-352
[26] Szabo S, Usadel KH (1982) Cytoprotection - organoprotection by somatostatin: gastric and hepatic lesions. Experientia 38: 254-256
[27] Turner HE, Nagy Z, Gatter KC, Esiri MM, Wass JAH, Harris AL (2000) Proliferation, bcl-2 expression and angiogenesis in pituitary adenomas: relationship to tumour behaviour. Br J Cancer 82: 1441-1445
[28] Usadel KH, Kessler H, Rohr G, Kusterer K, Palitzsch KD, Schwedes U (1986) Cytoprotective properties of somatostatins. Klin Wochenschr 64, Suppl 7: 59-63
[29] Wang DG, Johnston CF, Atkinson AB, Heaney AP, Mirakhur M, Buchanan KD (1996) Expression of Bcl-2 oncoprotein in pituitary tumors: comparison with c-myc. J Clin Pathol 49: 795-797
[30] Waśko R (1999) Apoptosis in the treatment of somatotropinoma and prolactinoma tumor types (in Polish). Thesis, AM Poznań
[31] Waśko R, Wołuń M, Warchoł JB (1999) Induction of apoptosis in cells of GH3 line by bromocriptine. Folia Histochem Cytobiol 37: 123-124
[32] Wiklund J, Wertz N, Gorski J (1981) A comparison of estrogen effects on uterine and pituitary growth and prolactin synthesis in F344 and Holtzmann rats. Endocrinology 109: 1700-1707
[33] Yin D, Kondo S, Takeuchi J, Morimura T (1993) Induction of apoptosis in rat somatotropin-secreting pituitary adenoma cells by bromocriptine. Oncol Res 5: 383-387
[34] Yin D, Kondo S, Takeuchi J, Morimura T (1994) Induction of apoptosis in murine ACTH-secreting pituitary adenoma cells by bromocriptine. FEBS Lett 339: 73-75
[35] Yin D, Tamaki N, Kokunai T, Yasuo K, Yonezawa K (1999) Bromocriptine-induced apoptosis in pituitary adenoma cells: relationship to p53 and bcl-2 expression. J Clin Neurosci 6: 326-331
[36] Yonezawa K, Tamaki N, Kokunai T (1997) Effects of bromocriptine and terguride on cell proliferation and apoptosis in the estrogen-stimulated anterior pituitary gland of the rat. Neurol Med Chir (Tokyo) 37: 901-906

Accepted October 31, 2003