FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 42, No. 4, 2004
pp. 245-247

Chromogranin A in pituitary adenomas: immunohistochemical detection and plasma concentrations

Marek Pawlikowski[1], Anna Gruszka[1], Maciej Radek[3] and Jolanta Kunert-Radek[2]

[1]Department of Experimental Endocrinology and Hormone Diagnostics, [2]Department of Clinical Endocrinology, Institute of Endocrinology, and [3]Department of Neurosurgery and Surgery of Peripheral Nerves, Medical University, Łódź, Poland

Abstract: Forty one pituitary adenomas excised surgically were immunostained to reveal pituitary hormones and chromogranin A (CgA). In 23 patients, plasma CgA concentration was determined before surgery by ELISA method. The CgA immunopositivity was found in 70.7% of investigated tumors. It was observed in all tumors of gonadotropinoma type and in the majority of null cell adenomas. Elevated (>18 U/L) plasma CgA concentration was observed in approx. a half of the examined patients, being more frequent in gonadotropinomas and null cell adenomas. It may have some, although limited, diagnostic value in these types of pituitary tumors.

Key words: Chromogranin A - Pituitary tumors - Gonadotropinomas - Null cell adenomas

Introduction

Chromogranin A (CgA) is a glycoprotein present in granules of neuroendocrine cells. It is abundantly expressed in neuroendocrine tumors, such as pheochromocytomas, gastrinomas, neuroblastomas, thyroid medullary cancers and carcinoid tumors and its plasma levels serves as marker of these tumors [2, 7, 10]. CgA immunopositivity is also an useful marker in histopathological diagnosis of neuroendocrine tumors [6]. It is also a marker of neuroendocrine differentiation of the non-endocrine cancers. CgA may be also expressed in pituitary adenomas [1, 4, 8, 13]. However, its relation to pituitary adenoma hormonal profile and outcome is not fully established, mainly because of limited series of investigated tumors. Plasma CgA concentrations were also investigated in patients with pituitary tumors but its usefulness as pituitary tumor marker is questionable [3, 5, 9]. The aim of the present study was to answer the following questions: (1) Does CgA immunopositivity depend on the hormonal immunotype of pituitary adenoma? (2) Is CgA tumor immunopositivity accompanied by elevated preoperative plasma CgA levels? (3) Can plasma CgA levels be a presurgical marker of pituitary adenomas (or of their specific types)?

Correspondence: M. Pawlikowski, Institute of Endocrinology, Medical University, Sterlinga 3, 91-425 Łódź, Poland;
e-mail: pawlikowski.m@wp.pl

Materials and methods

Forty one surgically excised pituitary adenomas were investigated. The diagnosis was based on preoperative clinical observation including pituitary hormone measurement in blood serum followed by histopathological and immunohistochemical examination of the excised tumor fixed in Bouin-Hollande fixative. The latter was performed on paraffin sections using the antibodies against pituitary hormones or their subunits. The following pituitary adenomas were included into study: 14 gonadotropinomas, 5 prolactinomas, 5 somatotropinomas co-secreting prolactin (somatoprolactinomas), 8 corticotropinomas (5 with Cushing disease and 3 without clinical symptomatology of hypercorticism), and 9 tumors immunonegative for all the investigated pituitary hormones (null cell adenomas). The adenomas immunopositive for FSH, LH or free alpha-subunit were all classified as gonadotropinomas following Trouillas *et al.* [14]. Chromogranin A immunoreactivity was investigated on paraffin sections using the prediluted polyclonal anti-human chromogranin A antibody (Dako, code H0085). The binding of primary antibody was detected using anti-rabbit IgG biotinylated goat antibody, streptavidin complex (Strept ABC/HRP, Dako) and 3,3'-diaminobenzidine.

Plasma concentrations of CgA were measured before surgery in 23 patients by using Chromogranin A ELISA Kit (DakoCytomation, Denmark).

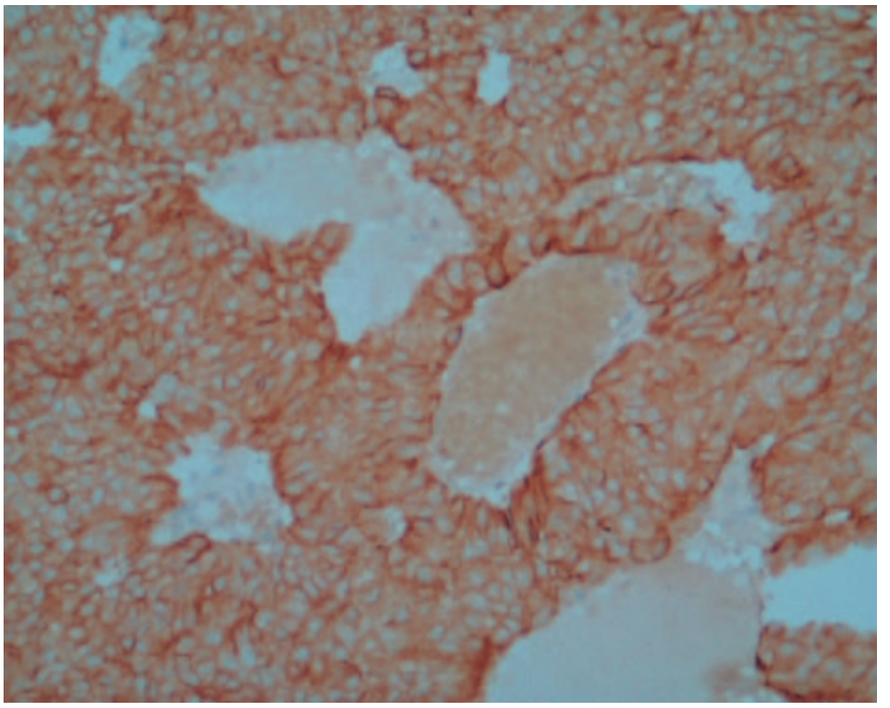

Fig. 1. Chromogranin A immunostaining in gonadotropinoma (free alpha subunit immunopositive adenoma) in 39 yrs old man. × 400.

Results and discussion

CgA immunopositivity was found in 29 out of 41 examined tumors (70.7%). The localization of immunostaining - on the cell periphery (Fig. 1) - was roughly similar in all CgA-immunopositive adenomas. The positive immunostaining was present in all (14/14) gonadotropinomas and in the majority of null cell adenomas (7/9). In corticotropinomas the chromogranin immunopositivity was observed in a half of tumors (4/8; including 3/5 of tumors causing the Cushing disease and 1/3 "silent" corticotropinomas). In contrast, CgA immunopositivity was rarely observed in mixed GH/PRL-secreting adenomas (somatoprolactinomas) and in prolactinomas (2/5 in both groups).

Before surgery, plasma CgA concentrations were elevated over the normal values established by the manufacturer of the ELISA kit (2-18 U/L) in 11/23 of the investigated patients. The elevated levels were noted in 6/9 patients with gonadotropinomas, 3/4 null cell adenomas,1/2 prolactinomas, 1/4 somatoprolactinomas and in none of 4 investigated patients with corticotropinomas. The mean concentration of plasma CgA was slightly higher in patients with chromogranin-immunopositive adenomas (26.5±25.5 U/L) than in those with chromogranin-immunonegative tumors (18.5±13.4 U/L) but the difference was statistically insignificant. In two cases, the plasma CgA levels were elevated in spite of the negative immunoreaction in tumors. The highest mean value was observed in patients with gonadotropinomas (37.5±35.4 U/L) and the lowest in somatoprolactinomas (8.4±6.2 U/L, see Fig. 2). However, the differences are not statistically significant probably because of limited number of determinations. Since only two measurements were done in prolactinomas, the mean value was not calculated for this subtype of tumor.

The data presented above confirm the earlier findings that pituitary adenomas can express chromogranin A. The CgA immunopositivity is a regular phenomenon in gonadotropinomas - which corroborates with earlier unpublished observations of J.Trouillas (personal communication) - and in null cell adenomas. Our data also agree with those of Colombo *et al.* [1] who found positive immunoscintigraphy with anti-chromogranin A antibody in the majority of non-functioning pituitary adenomas (9/11) but only in 1/4 growth hormone secreting tumors. The CgA expression in pituitary adenomas, as revealed by immunohistochemistry, is usually accompanied by the elevated CgA levels in plasma. However, the elevation is rather moderate and not regular. In some cases, the plasma CgA levels were within normal range in spite of marked chromogranin immunostaining in the tumor. In contrast, in two cases the plasma CgA concentrations were elevated in spite of the lack of immunostaining in the tumor. The latter finding can be explained either by the co-existence of the occult extra-pituitary neuroendocrine tumor or by the action of other factors which can influence the plasma CgA (for instance, the

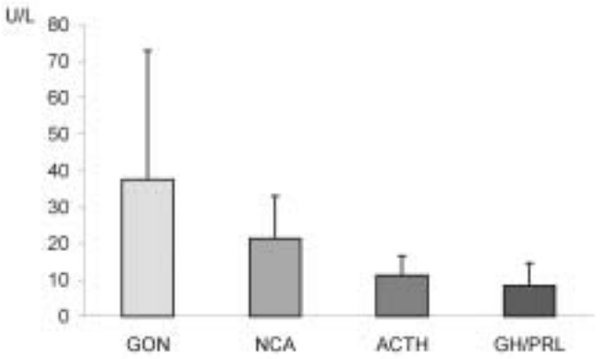

Fig. 2. Mean (SD) plasma chromogranin A concentrations in patients with different subtypes of pituitary adenoma. GON - gonadotropinoma; NCA- null cell adenoma; ACTH - corticotropinoma; GH/PRL - somatoprolactinoma.

sympathetic hyperactivity). Our findings support the view that the role of CgA plasma determination is of limited value as a pituitary adenoma marker [3, 5, 9]. However, the elevation of CgA, although moderate, occurs mainly in gonadotropinomas and null cell adenomas. These tumors clinically manifest as non-functioning and their early diagnosis is very difficult [11, 12]. Thus, even a moderate elevation of plasma CgA concentration should be a signal justifying search for a pituitary tumor.

The usefulness of plasma CgA monitoring after surgery in patients with chromogranin-immunopositive pituitary adenomas in order to evaluate the radicality of the operation and to predict tumor recurrence is very probable but needs further prospective studies.

Acknowledgements: This study was supported by the National Committee of Scientific Research (KBN), grant no. 3 PO5B 19622 for J.K-R.

References

[1] Colombo P, Siccardi AG, Paganelli G, Magnani P, Songini C, Buffa R, Faglia G, Fazio F (1996) Three step immunoscintigraphy with anti-chromogranin A monoclonal antibody in tumours of the pituitary region. Eur J Endocrinol 135: 216-221

[2] Ferrari L, Seregni E, Bajetta E, Marinetti A, Bombardieri E (1999) The biological characteristics of chromogranin A and its role as a circulating marker in neuroendocrine tumours. Anticancer Res 19: 3415-3428

[3] Gussi IL, Young J, Baudin E, Bidart JM,Chanson P (2003) Chromogranin A as serum marker of pituitary adenomas. Clin Endocrinol 59: 644-648

[4] Jin L, Zhang S, Bayliss J, Scheithauer B, Qian X, Kobayashi I, Stridsberg M, Lloyd RV (2003) Chromogranin A processing in human pituitary adenomas and carcinomas: analysis with region specific antibodies. Endocr Pathol 14: 37-48

[5] Kimura N, Miura W, Noshiro T, Mizunashi K, Hanew K, Shimizu K, Watanabe T, Shibukawa S, Sohn HE, Abe K, Miura Y, Nagura H (1997) Plasma chromogranin A in pheochromocytoma, primary hyperparathyroidism and pituitary adenoma in comparison with catecholamine, parathyroid hormone and pituitary hormones. Endocr J 44: 319-327

[6] Kimura N, Sasano N, Yamada R, Satoh J (1988) Immunohistochemical study of chromogranin in 100 cases of pheochromocytoma, carotid body tumour, medullary thyroid carcinoma and carcinoid tumour. Virch Arch A 413: 33-38

[7] Lamberts SW, Hofland LJ, Nobels FR (2001) Neuroendocrine tumor markers. Front Neuroendocrinol 22: 309-339

[8] Lloyd RV, Scheithauer BW, Kovacs K, Roche PC (1996) The immunophenotype of pituitary adenomas. Endocr Pathol 7: 145-150

[9] Nehar D, Deschamps M, Andrelli F, Claustrat B, Sassolas G (1999) Immunoradiometric assay of chromogranin A in patients with neuroendocrine tumors and pituitary adenomas. 9th Meeting of the European Neuroendocrine Association, Odense, September 3-7, 1999, Programme & Abstracts, Abstr 8-12

[10] Nehar D, Lombard-Bohas C, Olivieri S, Claustrat B, Chayvialle JA, Penes MC, Sassolas G, Borson-Chazot F (2004) Interest of chromogranin A for diagnosis and follow-up of endocrine tumours. Clin Endocrinol 60: 644-52

[11] Pawlikowski M (1997) The significance of the immunocytochemical investigations in diagnosis and prognosis of pituitary adenomas. Folia Histochem Cytobiol 35: 109-110

[12] Pawlikowski M, Kunert-Radek J, Radek A (2000) Gonadotropinoma - a frequent subtype of pituitary adenoma. Endokrynol Pol 51: 77-81

[13] Talan-Hranilovic J, Gnjidic Z, Sajko T, Vizner B, Vrkljan M (2000) Comparative immunohistochemical analysis of estrogen receptor and chromogranin A reactivity in plurihormonal human prolactinomas. Acta Med Croatica 54: 59-63

[14] Trouillas J, Girod C, Sassolas G, Claustrat B, Perrin G (1990) La cellule gonadotrope humaine normale et tumorale. Ann Endocrinol 51: 54-64

Accepted July 15, 2004