ONCOLOGY

Plasma Content of Soluble Fas Antigen in Patients with Adrenal Tumors and Tumor-Like Pathologies

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We compared plasma content of soluble Fas antigen (sFas) in 59 patients with tumors and tumor-like pathologies of the adrenal cortex and medulla and 60 healthy donors (control). The incidence and content of sFas in the plasma from patients with adrenal tumors was significantly higher than in healthy donors. A direct correlation was found between sFas content and patient's age. The maximum sFas concentrations were found in patients with pheochromocytoma and aldosterone-producing adenoma. In patients with adrenocortical cancer plasma content of sFas was lower than in patients with tumors of other morphological types. Plasma sFas content in patients with adrenocortical cancer directly correlated with the size of tumors. Our results suggest that sFas plays a role in the pathogenesis of primary adrenal tumors.

Key Words: *apoptosis; soluble Fas antigen; adrenal tumors*

Apoptosis is an important physiological process maintaining normal cell composition of organs and tissues. Apoptosis is responsible for removal of cells with damaged DNA, which prevents their fixation and formation of clones with carcinogenic mutations [14]. Fas receptor and its ligand FasL play a key role in apoptosis. Fas/APO-1/CD95 is a transmembrane glycoprotein belonging to the family of receptors for tumor necrosis factor and nerve growth factor [4,12]. It is expressed in the liver, kidneys, heart, thymus, thyroid gland, and ovaries. FasL is expressed in activated T lymphocytes and natural killer cells [10,13]; however constitutive expression of this cytokine was found in eye tissues [6] and testes [5]. The interaction of Fas receptors with FasL or monoclonal anti-Fas antibodies on the cell membrane triggers apoptosis of target cells.

There are membrane-bound (FasR) and soluble Fas receptors (sFas). sFas (trapping receptor) is a product of alternative splicing of full-length Fas mRNA. It inhibits the cytotoxic effect of FasL. Overexpression of sFas in various cells, including tumor cells, probably determines their resistance to factors regulating Fas-dependent apoptosis [8,10].

sFas content increases in patients with osteosarcoma [1], cancers of the thyroid gland [3], liver [12], ovaries [7,10] and thymus [9]. We found no published data on plasma levels of sFas in patients with adrenal tumors and tumor-like pathologies.

Here we studied the incidence and content of sFas in the plasma from healthy donors and patients with

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tumors and tumor-like pathologies of the adrenal cortex and medulla.

MATERIALS AND METHODS

We examined 59 patients (41 women and 18 men, 21-76 years) with primary adrenal tumors hospitalized at the Department of Surgical Endocrinology of M. F. Vladimirskii Moscow Regional Research-and-Clinical Institute and N. N. Blokhin Russian Oncology Center from December 1998 to February 2002. The history of the diseases (period between the appearance of symptoms to the start of therapy) varied from 2 months to 12 years. The patients received no antitumor therapy before examination. Clinical diagnoses were made by morphological assay of the adrenal glands after adrenalectomy. The following adrenal tumors were revealed (according to International Histological Typing of Endocrine Tumors [15]): adrenocortical adenoma (n=33), adrenocortical cancer (n=6), pheochromocytoma (n=10), adrenal cyst (n=5), myelolipoma (n=3), and ganglioneuroma (n=2).

The control group included 60 healthy donors (30 men and 30 women, 19-70 years).

Plasma sFas concentration was measured by sandwich enzyme-linked immunosorbent assay (ELISA) elaborated at the Laboratory of Clinical Biochemistry (N. N. Blokhin Russian Oncology Center) and Laboratory of Regulatory Proteins (M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry).

The blood for analyses was taken from the cubital vein of fasting patients with primary adrenal tumors and healthy donors at 8.00-9.00. After blood coagulation, the tubes were centrifuged at 500g for 10 min. The plasma was collected and stored at -20°C. The content of sFas was measured by enzyme immunoassay using monoclonal antibodies against Fas SA-8 — IgG1(κ) in a concentration of $(8.8\pm0.6)\times10^7$. The antibodies were sorbed overnight on Linbro plates in 0.05 M carbonate buffer (pH 9.6, 5.0 mg/ml) at 4°C. The plates were incubated with 1% bovine serum albumin (BSA) in phosphate buffered saline (pH 7.2) at 37°C for 1 h for inactivation of free binding sites. Plasma samples or serial dilutions of full-length recombinant Fas (40.0-0.15 ng/ml, positive control) were added to wells and the plates were incubated at 37°C for 1.5 h. After incubation the plates were thoroughly washed (6 times) with phosphate-saline buffer containing 0.1% Tween 20 (Sigma). This washout procedure was performed after each stage of the test. The plates were incubated with biotinylated antibodies against Fas SA-7 — IgG1(κ) $(9.52\pm1.4)\times10^8$ in washing buffer containing 0.1% BSA (12 μg/ml) for 2 h at 37°C or overnight at 4°C, respectively, and then with streptavidin peroxidase (Amersham) diluted in the washing buffer for 1 h (37°C). Freshly prepared 0.04% *o*-phenylenediamine in 50 mM citrate-phosphate buffer (pH 5.0) containing 0.03 H_2O_2 was added to wells for 15-20 min. The reaction was stopped by adding 50 ml 10% H_2SO_4. Optical density was measured on a MR 700 Microplate Reader spectrophotometer (Dynatech Labs) at 492 nm. sFas concentration in each plate was estimated by the calibration curve. The results were analyzed by SAS software.

Fluorometric assay was used to estimate 24-h urinary catecholamine excretion (epinephrine, norepinephrine, and dopamine). Cortisol and aldosterone concentrations in the serum and plasma were measured by enzyme immunoassay using Boehringer Mannheim kits.

RESULTS

In healthy donors no correlations were found between the incidence of sFas, its content, sex, and age (Table 1).

The incidence of sFas in patients was markedly higher than in healthy donors (88%, $p<0.01$). sFas content in patients varied from 0.6 to 24.7 ng/ml. In 60% patients sFas content surpassed its maximum concentration in healthy donors. The mean concentration of sFas in patients was 3.3±0.5 ng/ml, which significantly surpassed the control ($p<0.05$).

We found no sex differences in the incidence and content of sFas. sFas was detected in plasma samples from 13 male patients (72%); its content varied from 0.8 to 9.9 ng/ml (3.1±1.3 ng/ml). We revealed sFas in 95% female patients; its concentration varied from 0.6 to 24.7 ng/ml (2.9±1.0 ng/ml). A direct correlation was found between plasma sFas content and patient's age (R=0.9, $p<0.05$). No correlation was revealed between sFas concentration and duration of the disease.

The maximum sFas content was found in patients with pheochromocytoma (Table 1). It should be emphasized that in 4 patients the concentrations of sFas surpassed the maximum concentration in healthy donors. The incidence of sFas in female patients was 88%; its content varied from 0.6 to 6.7 ng/ml (2.0±0.8 ng/ml). sFas was detected in 1 of 2 patients with pheochromocytoma (75 years). In this patient sFas content was maximum (9.9 ng/ml). A direct correlation was found between sFas concentration in the plasma and age of patients with pheochromocytoma (R=0.8, $p<0.05$). No correlation was revealed between sFas concentration and duration of the disease.

Functional activity and the degree of tumor malignancy determine the clinical course and prognosis of pheochromocytoma. Pheochromocytoma cells secrete various biologically active substances, including dopamine, epinephrine, and norepinephrine. In patients with pheochromocytoma 24-h urinary excretion of

epinephrine varied from 34 to 600 nmol (150.3±138.5 nmol); in healthy donors this parameter was 11-44 nmol. In patients 24-h urinary norepinephrine excretion varied from 118 to 3369 nmol (1336.6± 897.2 nmol); in healthy donors this parameter was 47-236 nmol. No correlation was found between plasma sFas content in patients with pheochromocytoma and urinary excretion of catecholamines.

The presence of metastases in lymph nodes and distant organs is the major criterion for malignancy of pheochromocytoma. Invasion of tumor cells into surrounding tissues and vessels observed during histological assay does not indicate malignancy.

None of the patients had malignant pheochromocytoma (pheochromoblastoma). In 6 patients with benign pheochromocytoma sFas content was 2.7±1.5 ng/ml. In 2 female patients (41 and 43 years) morphological assay revealed signs of tumor malignancy (invasion of tumor cells into the capsule, fatty tissues, and vessels). In these patients plasma sFas content was 1.1 and 6.7 ng/ml, respectively.

The diameter of pheochromocytomas varied from 3.0 to 10.0 cm (5.3±1.3 cm). No correlation was found between sFas concentration and tumor size.

sFas was found in the plasma from patients with adrenocortical cancer. In these patients plasma sFas concentrations were lower than in patients with tumors of other morphological types (Table 1). It should be emphasized that only in 2 (33%) of 6 patients with adrenocortical cancer sFas content surpassed the control (1.9 and 8.0 ng/ml). No correlations were found between plasma sFas content, patient's age, and duration of the disease. The stage of adrenocortical cancer is the major criterion for the prognosis of this disease. In 5 of 6 patients the spread of adrenocortical cancer corresponded to stage III (T3N0M0). In 1 patient the spread of this tumor corresponded to stage IV (T3N0M1). In patients with stage III adrenocortical cancer plasma sFas concentration varied from 0.6 to 8.0 ng/ml (2.3±1.6 ng/ml). In the patient with spread adrenocortical cancer we found not only the primary tumor, but also numerous liver metastases. In this patient plasma sFas content was 1.9 ng/ml.

Hormonal activity of adrenocortical cancer serves as a marker of poor prognosis. In 3 patients these tumors were hormonally inactive. Clinical signs of Cushing's and virile syndromes were found in 2 and 1 patients, respectively. In female patients with hormonally inactive adrenocortical cancer (60, 67, and 72 years) sFas concentration did not differ from normal (0.7, 1.0, and 1.3 ng/ml). In 1 female and 1 male patients (58 and 33 years, respectively) with adrenocortical cancer and Cushing's syndrome sFas concentration was higher than in other patients of this group (1.9 and 8.0 ng/ml, respectively). The lowest sFas content was found in female patient with adrenocortical cancer and virile syndrome (43 years, 0.6 ng/ml).

The diameter of primary tumors in these patients varied from 4.5 to 13.5 cm (8.0±2.8 cm). Plasma sFas content directly correlated with tumor size (R=0.6, p>0.05).

In patients with aldosterone-producing adenoma the incidence of sFas was higher than in healthy donors (p<0.01, Table 1). sFas content in these patients underwent considerable variations. In 16 patients (57%) sFas content surpassed its maximum concentration in healthy donors.

sFas was revealed in all female patients with aldosterone-producing adenoma. Its concentration varied from 0.6 to 11.5 ng/ml (2.8±0.6 ng/ml). In male patients the incidence (70%) and average content of sFas (1.6±0.4 ng/ml, 0.8-3.7 ng/ml) were lower than in women (differences are insignificant, p>0.05). No correlations were found between plasma sFas content, patient's age, and duration of the disease. Plasma aldosterone concentration in patients with aldosterone-

TABLE 1. Incidence and Plasma Content of sFas in Patients with Tumors of the Adrenal Cortex and Medulla

Nosological type	Number of patients	Sex		Age, years	sFas incidence		sFas content, ng/ml	
		M	F		abs.	%	range	*M*±*m*
Adenoma								
aldosterone-producing	31	10	21	52.8±1.6	28	90	0.6-11.5	2.5±0.5
cortisol-producing	2	—	2	34, 42	1	50	—	24.7
Adrenocortical cancer	6	1	5	55.3±6.0	6	100	0.6-8.0	2.2±1.2
Pheochromocytoma	10	2	8	47.2±4.2	8	80	0.6-9.9	3.0±1.2
Cyst	5	1	4	43.2±3.6	4	80	0.8-4.9	2.4±1.0
Myelolipoma	3	—	3	31, 43, 54	2	67	1.4-2.2	1.4, 2.2
Ganglioneuroma	2	2	—	21, 23	1	50	—	3.6
Healthy donors (control)	60	30	30	38.1±7.4	17	28	0.6-1.3	0.8±0.3

producing adenoma was 0.08-4.20 nmol/liter (0.9±0.2 nmol/liter); in healthy donors this parameter was 0.14-1.24 nmol/liter. No correlation was revealed between plasma concentrations of sFas and aldosterone. Moreover, we found no correlation between the incidence of sFas, its content, and size of aldosterone-producing adenoma.

Since the number of patients with adrenal cyst was low, we did not analyze the dependence of sFas content and its incidence on clinical and morphological characteristics of the disease.

Our results indicate that the incidence of sFas in the plasma from patients with adrenal tumors is much higher than in healthy donors. In most patients sFas content surpasses its maximum concentration in healthy donors. A direct correlation was found between sFas concentration and patient's age. sFas concentration is highest in patients with pheochromocytoma and aldosterone-producing adenoma. In the plasma from patients with adrenocortical cancer sFas content is lower than in patients with adrenal tumors of other morphological types. Plasma sFas content in patients with adrenocortical cancer tended to correlate directly with the size of tumors.

These data suggest that sFas expression plays a role in the pathogenesis of adrenal tumors. However, low number of examined patients does not allow us to evaluate the clinical and prognostic significance of sFas measurements in these diseases.

REFERENCES

1. N. E. Kushlinskii, Yu. N. Solov'ev, I. V. Babkina, *et al*., *Byull. Eksp. Biol. Med*., **129**, No. 5, 583-586 (2000).
2. R. L. Baldwin, H. Tran, and B. Y. Karlan, *Gynecol. Oncol*., **74**, No. 2, 265-271 (1999).
3. F. Basolo, L. Fiore, A. Baldanzi, *et al*., *Lab. Invest*., **80**, No. 9, 1413-1419 (2000).
4. F. Beningi, S. Sacco, L. Aloe, and P. Chezzi, *Am. J. Pathol*., **153**, No. 5, 1377-1381 (1998).
5. O. Braendstrup, B. Bols, and L. Jensen, *APMIS*, **107**, No. 4, 431-436 (1999).
6. L. E. French, M. Hahne, I. Viard, *et al*., *J. Cell Biol*., **133**, 335-343 (1996).
7. L. Hefler, H. Mayerhofer, A. Nardi, *et al*., *Obstet. Gynecol*., **96**, No. 1, 65-69 (2000).
8. Y. Hiromatsu, T. Bednarczuk, and E. Soyejima, *Thyroid*, **9**, 341-345 (1999).
9. S. Kawanami, S. Mori, and H. Ueda, *Fukuoka Igaku Zasshi*, **91**, No. 5, 123-131 (2000).
10. R. Konno, T. Takano, S. Sato, and A. Yajima, *Clin. Cancer Res*., **6**, No. 9, 3576-3580 (2000).
11. S. H. Lee, M. S. Shin, H. S. Lee, *et al*., *Hum. Pathol*., **32**, No. 3, 250-256 (2001).
12. N. Mitsiades, V. Poulaki, S. Tseleni-Balafouta, *et al*., *Cancer Res*., **60**, No. 15, 4122-4129 (2000).
13. N. Mitsiades, V. Poulaki, G. Mastorakos, *et al*., *J. Clin. Endocrinol. Metab*., **84**, No. 8, 2924-2932 (1999).
14. F. G. Que and G. J. Gores, *Gastroenterology*, **110**, 1238-1243 (1996).
15. E. Solcia, G. Kloppel, and L. H. Sobin, *Histological Typing of Endocrine Tumours*, Geneva (2000), pp. 29-48.